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U.S. PATENT AND TRADEMARK OFFICE

In the application of:	)	Examiner: TBA
LEDBETTER et al.	)	Group Art Unit: TBA
Serial No.: 10/627,556	)	
Filed: July 26, 2003	)	
For: BINDING CONSTRUCTS	)	
AND METHODS OF USE	)	
THEREOF	)	

TRANSMITTAL LETTER

Mail Stop Petitions  
Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

Enclosed for filing for the above-referenced application please find:

1. Petition for Suspension of Rules under 37 C.F.R. § 1.183;
2. Declarations of:
  - A. Barbara J. Kielt;
  - B. James W. Collett, Ph.D. (includes Exhibit A, copy of U.S. application entitled "Binding Constructs and Methods of Use Thereof," now assigned U.S. Serial No. 10/627,556);
  - C. Michelle Hon;

CERTIFICATE OF MAILING  
(37 C.F.R. Section 1.8A)

I hereby certify that this paper (along with anything referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as First Class Mail in an envelope addressed to the Mail Stop Petitions, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

\_\_\_\_\_  
June 25, 2004  
Date of Deposit

Barbara J. Kielt  
Name of Person Mailing Paper  
\_\_\_\_\_  
Signature of Person Mailing Paper



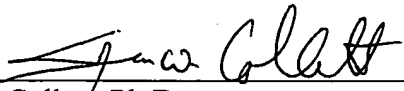
D. Mildred Pampena

E. Dale Stuckey, Jr.; and

F. John Norton.

The Commissioner is hereby authorized to charge Deposit Account No. 02-4553 in the amount of \$130.00. If this fee is incorrect, the Commissioner is hereby authorized to debit or credit Deposit Account No. 02-4553 accordingly.

Respectfully submitted,

  
\_\_\_\_\_  
James W. Collett, Ph.D.  
Reg. No. 46,636

BUCHANAN INGERSOLL P.C.  
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Patents  
49076.000004.CIP2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	Examiner: TBA
	)	
LEDBETTER et al.	)	Group Art Unit: TBA
	)	
Serial No.: 10/627,556	)	
	)	
Filed: July 26, 2003	)	
	)	
For: BINDING CONSTRUCTS AND	)	
METHODS OF USE THEREOF	)	

**PETITION FOR SUSPENSION OF RULES UNDER 37 C.F.R. § 1.183**

Attention: Office of Petitions  
Mail Stop Petition  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

Applicants hereby petition the Commissioner under 37 C.F.R. § 1.183 for a suspension of 37 C.F.R. § 1.10, which governs the filing of correspondence by "Express Mail." Applicants request this suspension of 37 C.F.R. § 1.10 to correct the filing date from July 26, 2003 to July 25, 2003 for the reasons set forth in this Petition and the accompanying

Declarations.

06/30/2004 TTRAN1 00000031 024553 10627556  
01 FC:1460 130.00 DA

**CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8**

I HEREBY CERTIFY that the Petition for Suspension of Rules under 37 C.F.R. § 1.183, along with supporting documentation, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Mail Stop Petitions, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, this 25th day of June, 2004.

Dated: June 25, 2004

By: Barbara J. Kiehl  
BARBARA J. KIELT

A Petition fee as set forth in 37 CFR 1.17(h) in the amount of \$130.00 is believed due. The Commissioner is authorized to debit the amount of \$ 130.00 from Deposit Account No. 02-4553 in payment of the Petition fee as set forth in 37 C.F.R § 1.17(h). In the event any additional amounts are due in connection with the filing of this Petition, the Commissioner is further authorized to debit the additional amounts.

Included with this Petition are the following Declarations:

1. **Declaration of Barbara Kielt**, administrative assistant/paralegal with Buchanan Ingersoll LLP (Kielt Decl.);
2. **Declaration of James W. Collett, Ph.D.**, Associate Attorney with Buchanan Ingersoll LLP (Collett Decl.);
3. **Declaration of Michelle Hon**, a Summer Associate with Buchanan Ingersoll LLP (Hon Decl.);
4. **Declaration of Mildred Pampena**, Manager of Networks and Communications with Buchanan Ingersoll PC (Pampena Decl.);
5. **Declaration of United States Post Office employee Dale Stuckey, Jr.** (Stuckey Decl.); and,
6. **Declaration of United States Post Office employee John Norton** (Norton Decl.).

This request for a correction of filing date has arisen from extraordinary circumstances that require intervention by the United States Patent and Trademark Office to correct an injustice that will result if the filing date is not corrected.

### **Background**

The application that is the subject of this Petition represents foundational technology of Trubion Pharmaceuticals, Inc., a start-up biotechnology company located in Seattle, Washington. The inventions described and claimed in the application relate to immunology and, more specifically, to immunotherapeutics for use in the treatment of a number

of serious disorders relating to the unwanted proliferation of cells, including cancers and autoimmune disorders, such as rheumatoid arthritis. Trubion began operations and received Series A funding in November 2002. It is now seeking Series B funding, and currently employs 32 people, all of whom are located in Seattle.

The subject application, titled “Binding Constructs and Methods of Use Thereof,” is 526 pages long. It relates to “Small Modular Immunotherapeutics” comprising a target binding domain polypeptide, a hinge region polypeptide and an effector function polypeptide comprising, for example, immunoglobulin CH2 and CH3 polypeptide sequences. The application describes over 100 different specific immunotherapeutic protein constructs, and contains 71 Figures in 75 sheets of drawings, 160 Examples, and 697 nucleotide and protein sequences (Collett Decl., Exhibit A). The application is a Continuation-in-Part of U.S. Serial No. 10/053,530, filed January 17, 2002 (entitled “Binding Domain-Immunoglobulin Fusion Proteins”), and also claims priority to U.S. Provisional Application No. 60/385,691 filed on June 3, 2002 (entitled “Recombinant Signaling Receptors for Tumor Gene Therapy”).

Trubion Pharmaceuticals has obtained significant results from use of constructs of the invention in preclinical animal studies. For example, company scientists have shown in preclinical studies that TRU015, a construct that is covered by this patent application, has an anti-disease function that is at least equivalent to, if not better than, that of Rituxan®, an anti-cancer therapeutic currently marketed by Genentech that generates sales of over \$1.3 billion annually. Trubion plans to file an Investigative New Drug application (an “IND”) this summer in order to pursue human clinical trials prior to marketing this new drug to patients.

**Request For Waiver Of Rule 10  
Due To Extraordinary Circumstances  
And In Order To Meet The Requirements Of Justice**

Rule 183 (37 C.F.R. § 1.183) provides in pertinent part:

In an extraordinary situation, when justice requires, any requirement of the regulations in this part which is not a requirement of the statutes may be suspended or waived by the Director or the Director's designee, *sua sponte*, or on petition of the interested party, subject to such other requirements as may be imposed.

Applicants respectfully request that the Commissioner suspend Rule 1.10 regarding Express Mail and award a filing date of July 25, 2003, the date the application was presented to the Post Office for filing but not accepted. Due to a combination of unforeseeable factors, this is a situation of extraordinary circumstances. Justice requires this earlier date because, without the correction of the filing date to July 25, 2003, PCT Application No. PCT/US02/01487, published July 25, 2002, Publication No. WO 02/056910 would become available for citation as a reference under 35 U.S.C. § 102(b), it having been published one year and one day before the official filing date of July 26, 2003 that was given to the present application.

Rule 10, entitled, "Filing of Correspondence by 'Express Mail,'" provides that, subject to certain exceptions not applicable here:

Any correspondence received by the U.S. Patent and Trademark Office (USPTO) that was delivered by the "Express Mail Post Office to Addressee" service of the United States Postal Service (USPS) will be considered filed with the USPTO on the date of deposit with the USPS, . . .

37 C.F. R. § 1.10(a)(1). The rule further provides in subsection (a)(2) that "[t]he date of deposit with USPS is shown by the 'date in' on the 'Express Mail' label or other official USPS notation." 37 C.F.R. § 1.10(a)(2). Additionally, subsection (b) states that "[c]orrespondence should be deposited directly with an employee of the USPS to ensure that the person depositing

the correspondence receives a legible copy of the ‘Express Mail’ mailing label with the ‘date-in’ clearly marked.” 37 C.F.R. § 1.10(b).

In this instance, several unforeseeable factors combined to block Applicants from obtaining a “date-in” of July 25, 2003, the date their application was presented to the Post Office for delivery to the PTO by Express Mail. Instead, Applicants were given a “date-in” of July 26, 2003. The first factor is the refusal of the Post Office to accept Applicants’ Express Mail packages, one of which contained the subject application. Although presented in person on July 25, 2004, the Post Office would not accept the packaged patent applications, stating that they were late because the hours of the main United States Post Office in San Diego (located near the airport on Midway Drive) had changed so that it was open only until 11:00 p.m., rather than midnight, its previous closing time.

As set forth in her Declaration accompanying this Petition, Barbara Kielt is a paralegal with Applicants’ law firm, Buchanan Ingersoll LLP. Ms. Kielt has been a legal administrative assistant since 1973, over 30 years, and has over eight years of direct patent prosecution experience. (Kielt Decl. ¶ 2). As noted in her Declaration, part of Ms. Kielt’s duties over her years of patent experience was to assist in the preparation and mailing of patent applications (Kielt Decl. ¶ 3). During this time she has known and on a number of occasions taken advantage of the fact that the San Diego’s main Post Office was open to accept Express Mail packages until midnight and had employees present to provide a “date-in” stamp on deposit any time before midnight (Kielt Decl. ¶¶ 4, 5 and 11). She reasonably relied on the fact that the main branch of the San Diego Post Office had been open until midnight for acceptance of Express Mail packages for years and that it would be open until midnight on July 25, 2003 (Kielt Decl. ¶¶ 1-5).

On the night of July 25, 2003, Ms. Kielt arrived at the Midway Post Office at approximately 11:40 p.m., well before the midnight cut-off for Express Mail (Kielt Decl. ¶ 11; Stuckey Decl. ¶¶ 3, 4; Norton Decl. P¶¶ 4,5). Ms. Kielt's arrival is confirmed and verified by the Declaration of Post Office employee Dale Stuckey, Jr. However, she was informed by Mr. Stuckey, who was behind the counter at the time, that the Post Office had changed its hours and that it only accepted packages presented by 11:00 p.m. (Kielt Decl. ¶ 12 ; Stuckey Decl. ¶ 4; Norton Decl. ¶ 5). Even though a Post Office employee, Mr. Stuckey, was working at that time, he would not accept the package (Kielt Decl. ¶ 12; Stuckey Decl. ¶ 4; Norton Decl. ¶ 5).

Another event on the night of July 25, 2003 was the unexpected shut down of the server that supports all of the computers and printers in the San Diego office of Buchanan Ingersoll LLP at approximately 10:00 p.m. (Collett Decl. ¶ 8; Hon Decl. ¶ 3; Kielt Decl. ¶ 8). The server shut down in the middle of making final typographical corrections and printing the U.S. application ultimately assigned Serial No. 10/627,556, which delayed the completion and printing of the application by at least 40 minutes (Collett Decl. ¶ 8; Kielt Decl. ¶ 8). It was unforeseeable to the personnel in the San Diego office of Buchanan Ingersoll that the computer server would shut down while completing and printing the subject U.S. application. Typically, if the Systems Department of Buchanan Ingersoll would shut down the computer system, it would send out notices in advance to allow people to plan for those occasions (Hon Decl. ¶ 5). This time, no notification was received until a few minutes before the server shut down (Collett Decl. ¶ 10). The computers and printers simply stopped working without advance warning (Hon Decl. ¶ 5; Collett Decl. ¶ 10) and were unavailable for over 30 minutes (Collett Decl. ¶ 8; Hon Decl. ¶ 3; Kielt Decl. ¶ 8). It was later learned that it was the practice of the Pittsburgh-based Systems Department of Buchanan Ingersoll to shut down the server nightly at 1:00 a.m. Eastern time

(Hon Decl. ¶ 6; Pampena Decl. ¶ 2) – which corresponds to 10:00 p.m. San Diego time – for maintenance. However, given that the intellectual property personnel in the San Diego office of Buchanan Ingersoll had only just recently joined the firm (Collett Decl. ¶ 11), and that this was the first time that any one in that group had been using the computers at 10:00 p.m. since joining the firm (Collett Decl. ¶ 12), they had no way of knowing about the daily practice of the Pittsburgh Systems Department and were, therefore, unable to plan ahead for the unavailability of their computers and printers. The San Diego office is the only office of Buchanan Ingersoll that is not in the Eastern time zone. Thus, the shut-down of the computers and printers on July 25 was not something that could have been prevented.

These circumstances combined to constitute a “Perfect Storm” of extraordinary and unforeseeable events. They were not due to simple inadvertence, and but for the change in hours at the Midway Post Office and the refusal of the Post Office to accept Applicants’ Express Mail package, despite the presence of an employee who could have, the package containing the patent application at issue would have been date stamped on July 25, 2003, the date it was presented to the Post Office pursuant to PTO rules.

Although “extraordinary circumstances” is not defined in the rules, the MPEP states that inadvertent failure to comply with requirements is not considered extraordinary. MPEP 500-61. Here, there was no inadvertent failure to comply with requirements.

Extraordinary circumstances have been found in a variety of cases. *See, e.g., In re Application of Hardee*, 223 U.S.P.Q. 1122 (Comr. 1984)(granting suspension of 37 C.F.R. § 1.48 under Rule 183 to allow removal of a co-inventor without co-inventor's consent upon showing proof of sole inventorship); *Helgott v. Director of the U.S. P.T.O.*, 209 F.3d 1328 (Fed.



Cir. 2000) (stating that a Rule 183 request should be granted to remedy mistakes made after PTO accidentally changed the agent's file reference number).

On the other hand, extraordinary circumstances are typically not found in cases where there is attorney neglect, ignorance of the law or ignorance of due dates. See, e.g., *In re: Reexamination of U.S. Patent No. 3,963,842*, 227 U.S.P.Q. 255 (Comr. 1985) (unawareness of patent laws and due dates is not an extraordinary circumstance, citing *Nugent v. Yellow Cab*, 295 F.2d 794 (7th Cir. 1961)); *Gustafson v. Strange*, 227 U.S.P.Q. 174 (Comr. 1985) (ignorance of 37 C.F.R. 1.8 is not extraordinary). Here, there was no attorney neglect, no ignorance of the law, and no ignorance of due dates. Indeed, as set forth in the Declaration of James W. Collett, Ph.D., Applicants' attorneys were working diligently on the preparation of this complicated patent application for several months, spending nearly 60 hours on its preparation in April and May 2003, over 80 hours working on drafts in June 2003, and over 300 hours finalizing various drafts in July 2003, including 59 hours during the first week of July, 75 hours during the second week in July, 108 hours during the third week in July, and 98 hours during the week of July 20, the week it was due to be filed.

The instant situation is different from that in *Nitto Chemical Industry v. Commr*, 39 U.S.P.Q.2D 1778 (D. D.C. 1994), where, on review of the Commissioner's denial of Nitto's petition to waive Rule 10, the court determined that there were no extraordinary circumstances justifying the waiver. In that case, an attorney who had finished two patent applications reportedly gave them to a secretary who prepared two Express Mail packages. The secretary then gave them to an assistant manager for the firm's mail contractor (Betancourt) who signed the Express Mail certifications attesting that the packages were to be deposited with the USPS for delivery to the PTO via Express Mail service. Betancourt then returned the certifications to

the attorney and gave the packages to another employee of the mail contractor (Perez) to deliver to the Post Office. The Declaration of Perez stated that he thought the mail he was given contained only first class mail but did not know whether it contained any Express Mail packages. All of the first class mail delivered by Perez to the Post Office was ultimately delivered to the appropriate destinations, but the PTO never received any of eight separate Express Mail packages, including the two at issue in the case.

The Commissioner denied applicant's request to waive the Rule 10 filing requirements because "there was no persuasive evidence that the original application papers were, in fact, deposited with the USPS on February 29, 1991" and because the "mishandling of the Express Mail packages amounted to an 'oversight that could have been prevented by the exercise of ordinary care or diligence'." *Nitto*, 39 U.S.P.Q.2d at 1778. On appeal, the district court affirmed denial of the petition. First, the district court found unpersuasive Nitto's argument that it had provided documentary evidence equivalent to a certificate of mailing because the Rule 10 requirement that a certificate of mailing be signed and dated by the person mailing the application was not met. In fact, the person who signed the certificate (Betancourt) did not mail them, but instead gave the packages to someone else (Perez) to mail.

The district court also ruled that it was not "unreasonable for the Commissioner to conclude that Nitto's evidence was not as probative of the mailing date of the packages as an Express Mail receipt." *Nitto*, 39 U.S.P.Q.2d at 1778. Nitto's evidence included an affidavit of Betancourt, who stated that he had put the Express Mail packages in the evening mailbag that he said he handed to Perez, and an affidavit of Perez stating that he deposited the entire contents of the mailbag at the Post Office.

Finally, the district court held that Nitto did not submit any evidence showing that the inventions claimed in the patent applications at issue would be denied patentability and, consequently, the Commissioner had no basis on which to determine whether "justice required" him to award the earlier filing date. 39 U.S.P.Q.2d at 177.

The present situation is distinguishable in every respect from that of *Nitto*.

1. First, in *Nitto* there was no evidence that the packages were deposited in Express Mail (instead, Perez's Declaration said that he had no specific recollection of taking Express Mail packages to the Post Office on the date in question) and the packages never arrived at the PTO. Here, the packages were deposited in Express Mail and the packages did arrive at the PTO. Applicants have provided Declarations from two Post Office employees and Ms. Kielt demonstrating that on July 25, 2003, Ms. Kielt presented two Express Mail packages addressed to the PTO at the Post Office – one containing the subject patent application – and attempted to deposit them for Express Mail delivery (Kielt Decl. ¶¶ 11-13; Stuckey Decl. ¶¶ 3 and 4; Norton Decl. ¶¶ 5 and 6). Additionally, the Declarations conclusively establish that the same packages with the same contents did arrive at the PTO, as further evidenced by the fact that a serial number has been assigned for the two applications contained in the two packages;
2. Second, in *Nitto*, the court emphasized that one of the reasons that Express Mail receipts are probative is that "a disinterested USPS employee" signs them. *Nitto*, 39 U.S.P.Q.2d at 1780. Here, applicants have provided the signed Declarations of disinterested Post Office employees in support of their Petition;
3. Third, in *Nitto*, the person who signed the certificate of mailing was not the person who allegedly delivered the packages to the Post Office. In the present case, the person who signed the certificate of mailing, Ms. Kielt, did deliver the package to the Post Office (Kielt Decl. ¶¶ 11-13; Stuckey Decl. ¶¶ 3 and 4; Norton Decl. ¶¶ 5 and 6); and,
4. Fourth, unlike *Nitto*, justice requires the earlier filing date because the patentability of the inventions could be adversely affected if this Petition is not granted.

In summary, the circumstances in the present case are a "Perfect Storm" of the unexpected convergence of extraordinary and unforeseeable events noted above and were not due to simple inadvertence. The change in hours at the Midway Post Office, so that it did not accept Express Mail packages after 11:00 p.m., the fact that the server shut down in the middle of printing the U.S. application, and the fact that the San Diego office of Buchanan Ingersoll had

recently added personnel who were unaware that the server routinely shut down at 10:00 p.m. each night, all combined to prevent Ms. Kielt from obtaining a date-in stamp of July 25, 2003, even though the patent application was presented to the U.S. Post Office on that day for filing pursuant to PTO rules.

Justice requires this earlier date because, without the correction of the filing date to July 25, 2003, PCT Application No. PCT/US02/01487, published July 25, 2002 (Publication No. WO 02/056910) would become available for use as a reference under 35 U.S.C. § 102(b), it having been published one year and one day before the filing date given to the present application (currently listed as July 26, 2003, but sought to be corrected to July 25, 2003 by this Petition).

The present application is a continuation-in-part of U.S. Serial No. 10/053,530, filed January 17, 2002. The above-noted published PCT Application (PCT/US02/01487) – which becomes available as a 102(b) reference unless the filing date is corrected – corresponds to the U.S. '530 application and has the same specification. One of the advantages of filing a continuation-in-part application rather than a separate application is that the parent application may not itself be used as prior art against the CIP application. Thus, if the filing date is not corrected to the date the application was presented to the Post Office, as requested by Applicants, the present application is effectively turned into an independent application due to the publication of the PCT application corresponding to its parent. The advantage of filing the present application as a CIP application would be lost and the parent application could be used as a 102(b) reference against the present application.

**Conclusion**

Based upon the foregoing information and Declarations, Applicants submit that the failure to obtain a July 25, 2003 filing date for the above-referenced patent application was an extraordinary situation that requires the suspension of rule 37 C.F.R. § 1.10 by the Commissioner in the interest of justice. Applicants respectfully request that the filing date of the above-identified application be corrected to July 25, 2003.

Dated: June 25, 2004

Respectfully submitted,

By: James W. Collett  
James W. Collett, Esq., Ph.D  
Reg. No. 46,646

## **DECLARATION OF BARBARA J. KIELT**

I, Barbara J. Kielt, declare as follows:

1. I am employed as a paralegal in the Intellectual Property Group of the San Diego office of Buchanan Ingersoll LLP, located at 401 West A Street, Suite 1900, San Diego, California. On July 25, 2003, I was employed by Buchanan Ingersoll LLP as an administrative assistant in the Intellectual Property Group of the San Diego Office of Buchanan Ingersoll LLP.

2. Prior to working at Buchanan Ingersoll, I held positions as an intellectual property administrative assistant since 1996, with Brobeck, Phleger & Harrison LLP, Lyon & Lyon LLP, InterVu, Inc., Akamai Technologies, Inc. and Kintera, Inc. I have been a legal administrative assistant since 1973.

3. One of my responsibilities as an intellectual property administrative assistant or paralegal is to prepare and mail patent applications, and I always mail these patent applications by Express Mail through the United States Postal Service.

4. In my employment as an intellectual property administrative assistant over the last several years, I have personally deposited patent applications by Express Mail, and had U.S. Post Office employees present to provide a "date-in" stamp, at the U.S. Post Office, located at 2585 Midway Drive, San Diego between 11:00 p.m. and 12:00 midnight.

5. During these past 8 years as an intellectual property administrative assistant, I have never known the Post Office to change its hours and relied upon the fact that the 2585 Midway Drive Post Office was open until 12:00 midnight for acceptance of Express Mail packages.

6. On Friday, July 25, 2003, I assisted in the preparation of two patent applications: (1) a U.S. Application entitled, "Binding Constructs and Methods of Use Thereof," now assigned U.S. Serial No. 10/627,556; and (2) a PCT application entitled, "Binding Domain-Immunoglobulin Fusion Proteins," now assigned PCT Serial No. PCT/US02/2491, which were to be filed with United States Patent and Trademark Office by Express Mail on that date.

7. The PCT application was ready to be filed by early afternoon on July 25, 2003, and was placed in a sealed package with an Express Mail label filled out at that time, but was held until the U.S. application was ready to be filed so both applications could be taken to the Midway Post office at the same time and mailed by Express Mail.

8. At 10:00 p.m. on July 25, 2003, while we were trying to complete and print the final version of the U.S. patent application, the server supporting all of the computers and printers in the San Diego office of Buchanan Ingersoll disconnected, and we lost all access to the document and were unable to continue. It was not until after sometime after 10:30 p.m. that I was able to access the server, and further time was required to reboot, log on and open the document, leading to the loss of at least 40 minutes due to the server shut-down.

9. All work on the U.S. application, which was 526 pages long and had an additional 75 sheets of drawings, was completed and the patent application was separately packaged and addressed to the United States Patent and Trademark Office to the attention of the appropriate Mail Stop and I immediately left the office at approximately 11:25 p.m..

10. I drove to the U.S. Post Office, located at 2585 Midway Drive, San Diego, CA, in order to deposit these two applications by Express Mail with an employee of the U.S. Post Office before midnight on July 25, 2003.

11. I entered the Post Office at approximately 11:40 p.m. A U.S. postal clerk named Dale Stuckey whom I recognized from several other times I was at this Post Office to make late evening Express Mail filings, was standing at the window where mail is accepted by the Post Office. A bar had been pulled down at this window and it appeared to be closed.

12. Mr. Stuckey informed me that the Midway Post Office had changed its closing hours to 11:00 p.m. and refused to accept my Express Mail packages. When I asked him when the change had occurred, he said that it had been at least several months.

13. I was unable to deposit my two packages containing the patent applications by Express Mail because of Midway Post Office's change of closing time and, therefore, did not obtain a U.S. Patent & Trademark Office filing date of July 25, 2003.


14. After the Post Office's refusal to accept the two Express Mail packages on Friday, July 25, 2003, I attempted unsuccessfully to reach Dr. Collett to obtain instructions.

15. On Saturday morning, July 26, 2003, I contacted Dr. Collett by telephone and explained what had happened at the Post Office. Dr. Collett instructed me to deposit the packages containing the two patent applications by Express Mail with the U.S. Postal Service on Saturday, July 26, 2003.

16. The two patent applications and the entire contents of the two packages deposited with the U.S. Postal Service on Saturday, July 26, 2003 are the same and identical to those prepared and packaged Friday, July 25, 2003.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: June 25, 2004.

  
Barbara J. Kielt



**DECLARATION OF JAMES W. COLLETT, PH.D.**

I, James W. Collett, Ph.D., declare as follows:

1. I am employed as an associate in the Intellectual Property Group of the law firm of Buchanan Ingersoll LLP, located at 401 West A Street, Suite 1900, San Diego, California.

2. I have been employed in this position since April of 2003.

3. Prior to this position, I have held positions as an intellectual property attorney for the preceding five years, including positions with Amylin Pharmaceuticals, Inc. and Sheldon & Mak LLP.

4. My responsibilities as an associate in the Intellectual Property Group of Buchanan Ingersoll LLP include the preparation of patent applications and the supervision of Barbara J. Kielt, then an administrative assistant in the Intellectual Property Group of Buchanan Ingersoll LLP.

5. On Friday, July 25, 2003, I assisted in the completion of two patent applications: (1) a U.S. Application entitled, "Binding Constructs and Methods of Use Thereof," now assigned U.S. Serial No. 10/627,556; and (2) a PCT application entitled, "Binding Domain-Immunoglobulin Fusion Proteins," now assigned PCT Serial No. PCT/US02/24918. I supervised Ms. Kielt in preparing these two patent applications to be filed with United States Patent and Trademark Office by Express Mail on that date.

6. The completion and preparation for filing on July 25, 2003, was the culmination of several months of work on the U.S. application. Applicants' attorneys spent nearly 60 hours on its preparation in April and May 2003, over 80 hours working on drafts in June 2003, and over 300 hours finalizing various drafts in July 2003, including 59 hours during the first week of July, 75 hours during the second week in July, 108 hours during the third week in July, and 98 hours during the week of July 20, the week it was due to be filed.

7. The PCT application was ready to be filed by early afternoon on July 25, 2003, but was held until the U.S. application was ready to be filed so both applications could be mailed by Express Mail at the Midway Post office at the same time.

8. At 10:00 p.m. on July 25, 2003, while we were trying to complete and print the final version of the U.S. patent application, the server supporting all of the

computers and printers in the San Diego office of Buchanan Ingersoll LLP disconnected, and we lost all access to the document. It was not until sometime after 10:30 p.m. that we were able to access the server. Further time was required to reboot, log on and open the document, leading to the loss of at least 40 minutes due to the server shut-down. A copy of the U.S. patent application, which consists of 526 pages and 75 sheets of drawings, is attached hereto as Exhibit A.

9. The server is located in the main office of Buchanan Ingersoll PC, which is in Pittsburgh, Pennsylvania, and we had no access to the server or control over it.

10. Prior to July 25, 2003, I had received several notifications from the Systems Department in the Pittsburgh office of Buchanan Ingersoll PC that the server would be shut down for maintenance at various times, typically over a weekend. I received no such notification that this would happen on Friday, July 25, 2003, until a few minutes before the server shut down.

11. Before April, 2003, the San Diego office of Buchanan Ingersoll LLP did not have any intellectual property staff. In April, 2003, Bradford J. Duft joined as a shareholder, and Ms. Kielt as an administrative assistant, in the Intellectual Property Group in the San Diego office. As noted above, I joined the San Diego office of Buchanan Ingersoll LLP as an Associate in the Intellectual Property Group in April 2003.

12. The first time that I or anyone else in the Intellectual Property Group in the San Diego office of Buchanan Ingersoll LLP had tried to use the computers or printers between 10:00 and 10:30 p.m. was July 25, 2003. Consequently, before that date, no one in the Intellectual Property Group in the San Diego office was aware of, what I later learned from Michelle Hon, a Summer Associate with Buchanan Ingersoll LLP, were nightly shut-downs of the server controlling all of the computers and printers in the San Diego office.

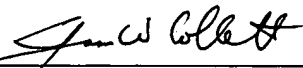
13. All work on the U.S. application was completed in the Buchanan Ingersoll LLP San Diego office and the application was ready to file not later than about 11:25 p.m. Each patent application and its corresponding transmittal papers had been placed in separate packages by Ms. Kielt and each package was addressed to the United States Patent and Trademark Office to the appropriate Mail Stop. Ms. Kielt left at that

time to drive to the Midway Drive Post Office to deposit the two packages by Express Mail.

14. Ms. Kielt contacted me the next morning, Saturday, July 26, 2003, and explained what had happened at the Post Office the evening before, namely, that a Post Office employee was at the Post Office when Ms. Kielt arrived with the packages but that he had refused to accept them, stating that the branch had changed its Express Mail deposit cut-off time from midnight to 11:00 p.m. I instructed Ms. Kielt to deposit the two packages by Express Mail with the U.S. Postal Service on Saturday, July 26, 2003.

I declare under penalty of perjury that the foregoing is true and correct to the best of my knowledge.

Dated: June 25, 2004.

  
\_\_\_\_\_  
James W. Collett, Ph.D.

## **EXHIBIT A**

Copy of U.S. Application entitled, "Binding Constructs and Methods of Use Thereof,"  
now assigned U.S. Serial No. 10/627,556.

## BINDING CONSTRUCTS AND METHODS FOR USE THEREOF

### CROSS REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. Serial No. 10/053,530, filed  
5 January 17, 2002, which is incorporated herein by reference in its entirety and which claims  
the benefit of priority of U.S. Provisional Application Serial No. 60/367,358 (formerly U.S.  
Serial No. 09/765,208, filed January 17, 2001), also incorporated herein by reference in its  
entirety and for which a Petition to Convert a Non-Provisional Application to a Provisional  
Application was filed on January 16, 2002, and granted on June 6, 2002. This application  
10 also claims the benefit of priority of U.S. Provisional Application No. 60/385,691 filed on  
June 3, 2002, and entitled "Recombinant Signaling Receptors for Tumor Gene Therapy,"  
which is also incorporated herein by reference in its entirety.

### FIELD OF THE INVENTION

15 The present invention relates generally to compounds having various utilities  
including uses for research, diagnostics, and therapy, for example, immunotherapy.  
Compounds of the invention include immunologically active proteins and protein  
conjugates. Such proteins include recombinant or engineered binding proteins such as, for  
example, binding domain-immunoglobulin fusion proteins, which may include single chain  
20 Fv-immunoglobulin fusion proteins and compounds containing single chain Fv-  
immunoglobulins. The present invention also relates to compositions and methods for  
treating conditions, diseases and disorders that would improved, eased, or lessened from the  
administration of, for example, polypeptide and/or nucleic acid constructs of the invention,  
including, for example, malignant conditions and B cell disorders, including diseases  
25 characterized by autoantibody production and/or inflammation.

### BACKGROUND OF THE INVENTION

The immune system is one of the most complex of the body's many intricate  
systems. A vast and complicated arrangement made up of many different types of cells and  
30 involving many different kinds of molecules, the human immune system allows the body to

respond to foreign invaders such as bacteria, viruses, and other infectious agents, as well as foreign material such as pollen. In general, the human immune system is divided into two main parts, antibody-mediated immunity (also called “humoral” or “circulating” immunity) and cell-mediated immunity, both of which are managed by lymphocytes. Lymphocytes are one of the five kinds of white blood cells (leukocytes) circulating in the blood. There are several kinds of lymphocytes, each with different functions to perform. The most common types of lymphocytes are B lymphocytes (B cells), which are responsible for making antibodies, and T lymphocytes (T cells). Cells of the immune system not only include T cells and B cells, but also Natural Killer Cells, granulocytes (or polymorphonuclear (PMN) leukocytes), macrophages, and dendritic cells. The humoral system is managed by B cells with help from T cells and deals with infectious agents in the blood and tissues of the body. The cell-mediated system is managed by T cells and deals with cells of the body that have been infected.

An antigen is a substance, usually macromolecular, that stimulates or induces an immune response. Because of its complex macromolecular structure, a single microorganism consists of multiple antigens (*e.g.*, surface structures such as cell wall components, fimbriae, flagella, *etc.*, or extracellular proteins, such as toxins or enzymes produced by the microorganism). The coat proteins and some of the envelope proteins of animal viruses are also usually antigenic. A host is generally able to respond specifically to antigens that come into contact with components of its immune system. Both the antibody-mediated immunity and cell-mediated immunity systems involve complex interrelationships that allow them to mount immune reactions to almost any antigen. In other words, the immune system is able to recognize foreign substances (antigens) that stimulate the system to produce antibody-mediated immunity, cell-mediated immunity, or both.

The immune system complex is constituted by a variety of different cell types and organs disseminated throughout the body. These include the primary lymphoid organs, *i.e.*, the bone marrow and the thymus. All the cells of the immune system are initially derived from the bone marrow in a process called hematopoiesis. During hematopoiesis bone marrow-derived stem cells differentiate into either mature cells of the immune system (“B” cells) or into precursors of cells that migrate out of the bone marrow to mature in the

thymus (“T” cells). In addition to red blood cells, platelets, and B cells, the bone marrow also produces Natural Killer cells, granulocytes, and immature thymocytes. The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus where they mature and are then released into the bloodstream.

The immune system complex also includes secondary lymphoid organs, *e.g.*, the spleen, the lymph nodes, *etc.*, as well as a circulatory system that is separate from blood vessels. The spleen, made up of B cells, T cells, macrophages, dendritic cells, Natural Killer cells, and red blood cells, is an immunologic filter of the blood. In addition to capturing antigens from the blood that passes through the spleen, migratory macrophages and dendritic cells bring antigens to the spleen via the bloodstream. An immune response is initiated in the spleen when macrophages or dendritic cells present the antigen to the appropriate B or T cells, and B cells become activated and produce large amounts of antibody. Lymphatic vessels and lymph nodes are the parts of a special circulatory system that carries lymph, a transparent fluid containing white blood cells, chiefly lymphocytes. Lymph bathes the tissues of the body, and is then collected in lymphatic vessels. Lymph nodes dot the network of lymphatic vessels and function as an immunologic filter for lymph when afferent lymph ducts bring lymph-containing antigens into the node. Composed mostly of T cells, B cells, dendritic cells, and macrophages, the lymph nodes drain fluid from most tissues. Antigens are filtered out of the lymph in the lymph node before the lymph is returned to the circulation. Macrophages and dendritic cells that capture antigens also present these foreign materials to T and B cells in the lymph nodes, resulting in the stimulation of B cells to develop there into antibody-secreting plasma cells. Antibodies leave the lymph node by the efferent ducts that empty into the blood stream. Lymphocytes can also leave the node by the efferent duct and travel to other sites in the lymphatic system or enter into the blood circulation. A single lymphocyte completes a circuit through the circulating blood and lymphatic systems once every 24 hours.

Tonsils, adenoids, Peyer’s patches, and the appendix are also lymphoid tissues. Peyer’s patches (masses of lymphocytes) are similar to the tonsils and are found throughout the body, especially in the mucous linings of the digestive and respiratory tracts.

It is the function of the phagocytic cells found in Peyer's patches and other lymphatic aggregate follicles to defend the body against, for example, inadequately digested food particles crossing the gut wall and entering the blood, and to attack unwanted foreign invaders while they are still in the bowel.

5                   The major function of B cells is the production of antibodies in response to foreign proteins of bacteria, viruses, and tumor cells. T cells are usually divided into two major groups, namely, the cytotoxic T lymphocytes ("Tc" cells or CTLs) and the helper T cells ("Th" cells or T helper cells). Th cells, also referred to as CD4+ T cells, function to augment or potentiate immune responses by the secretion of specialized factors that activate  
10 other white blood cells to fight off infection. They enhance the production of antibodies by B cells. Tc cells, also called CD8+ T cells, can directly kill certain tumor cells, viral-infected cells, and sometimes parasites. Tc cells are also important in down-regulation of immune responses. Both types of T cells often depend on the secondary lymphoid organs (the lymph nodes and spleen) as sites where activation occurs, but they are also found in  
15 other tissues of the body, including the liver, lung, blood, and intestinal and reproductive tracts.

Natural Killer cells, often referred to as NK cells, represent another type of lymphocyte and are similar to the Tc cell subset. They function as effector cells that directly kill certain tumors such as melanomas and lymphomas, and viral-infected cells. They are  
20 called "natural" killers because, unlike cytotoxic T cells, they do not need to recognize a specific antigen before carrying out their function. While NK cells, unlike the Tc cells, kill their targets without prior activation in the lymphoid organs, NK cells activated by Th cell secretions will kill tumor or viral-infected targets more effectively. NK cells target tumor cells and protect against a wide variety of infectious microbes. In several immunodeficiency  
25 diseases, including AIDS, Natural Killer cell function is abnormal. Natural Killer cells may also contribute to immunoregulation by secreting high levels of influential lymphokines. Some NK cells have surface receptors (FcγRIII, also called CD16) for the Fc portion of the IgG antibody. They bind to target cells through receptors for the Fc portion of an antibody that has reacted with antigen on a target cell. This type of cell-mediated immunity is called  
30 antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells may also have receptors



for the C3 component of complement, another immune defense system, and therefore recognize cells that are coated with C3 as targets. ADCC is thought to be an important defense against a variety of parasitic infections caused, for example, by protozoa and helminths.

5           Although small lymphocytes look identical, they can be distinguished by molecules carried on their cell surface. Not only do such markers distinguish between B cells and T cells, they distinguish among various subsets of cells that behave differently. Every mature T cell, for instance, carries a marker known as T3 (or CD3). In addition, most helper T cells carry a T4 (CD4) marker, a molecule that recognizes Class II major  
10   histocompatibility complex ("MHC") antigens. A molecule known as T8 (CD8), which recognizes Class I MHC antigens, is found on many suppressor/cytotoxic T cells.

          Another group of white blood cells collectively referred to as granulocytes, or polymorphonuclear leukocytes (PMNs), is composed of three cell types. These cells, neutrophils, eosinophils, and basophils are important in the removal of bacteria and parasites  
15   from the body. Neutrophils migrate through capillary walls and into infected tissue where they kill invaders (*e.g.*, bacteria) and then engulf the remnants by phagocytosis. Eosinophils are cytotoxic, releasing the contents of their granules on an invader. Basophils leave the blood and accumulate at the site of an infection or other inflammation and discharge the contents of their granules, releasing a variety of mediators such as histamine, serotonin,  
20   prostaglandins and leukotrienes that, for example, increase blood flow to the area. Mediators released by basophils also play an important part in some allergic responses such as hay fever and anaphylactic responses to insect stings.

          Monocytes are large phagocytic white blood cells released from the bone marrow into the blood circulation. When a monocyte enters tissue, it develops into a  
25   macrophage. Macrophages are also large, phagocytic cells that engulf foreign material (antigens) that enter the body, as well as dead and dying cells of the body. Macrophages are important in the regulation of immune responses, and are often referred to as scavengers, or antigen-presenting cells (APCs) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This  
30   is one of the important first steps in the initiation of an immune response. Stimulated

macrophages exhibit increased levels of phagocytosis and also secrete Interleukin-1 (IL-1), a product that helps to activate B cells and T cells.

Dendritic cells also originate in the bone marrow and function as APCs. They are usually found in the structural compartment of lymphoid organs such as the thymus, lymph nodes and spleen, but are also found in the bloodstream and other tissues. It is believed that dendritic cells capture antigen or bring it to the lymphoid organs where an immune response is initiated.

Important features of the immunological system relevant to host defense and/or immunity to pathogenic microorganisms include specificity, memory, and tolerance. It is understood, for example, that an antibody or reactive T cell will react specifically with the antigen that induced its formation; it will not react with other antigens. Generally, this specificity is of the same order as that of enzyme-substrate specificity or receptor-ligand specificity, although cross-reactivity is possible. The specificity of the immune response is explained by clonal selection. During the primary immune response, a specific antigen selects a pre-existing clone of specific lymphocytes and stimulates its activation, proliferation and differentiation. It is also understood that once the immune system has responded to produce a specific type of antibody or reactive T cell, it is capable of producing more of the antibody or activated T cell more rapidly and in larger amounts; this is called the secondary (or memory) response. It is also recognized that an animal generally does not undergo an immunological response to its own (potentially-antigenic) components. The animal is said to be tolerant, or unable to react to its own potentially antigenic components. This ensures that under normal conditions, an immune response to “self” antigens (called an autoimmune response) does not occur. Tolerance is brought about in a number of ways, but in essence the immune system is able to distinguish “self” components from “non-self” (foreign) antigens; it will respond to “non-self” but not to “self”. Sometimes in an animal, tolerance can be “broken”, which may result in an autoimmune disease.

The biological activities of the antibody-mediated and cell-mediated immune responses are different and vary from one type of infection to another. There are several classes or types of antibodies (and subclasses of various types) involved in antibody-mediated immunity. All of the classes of antibodies that are produced in response to a

specific antigen react stereochemically with that antigen and not with other (different) antigens. The host has the genetic capacity to produce specific antibodies to thousands of different antigens, but does not do so until there is an appropriate (specific) antigenic stimulus. Due to clonal selection, the host produces only the homologous antibodies that will react with that antigen which, as noted above, are found in blood (plasma), lymph, and many extravascular tissues. Once the antibody-mediated immunity response occurs following interaction of B lymphocytes with antigen and their differentiation into antibody-secreting plasma cells, the secreted antibody binds to the antigen which, in turn, results in its neutralization or elimination from the body.

Cell-mediated immunity, on the other hand, is mediated by specific subpopulations of T-lymphocytes called effector T cells that exist in precursor form as “resting T cells” (pT cells). These cells bear receptors for specific antigens and recognize these antigens on the surfaces of other cells. Stimulation with that antigen results in T cell activation. T cells enlarge, enter into a mitotic cycle, reproduce and develop into effector T cells whose activities are responsible for this type of immunity. They also develop into clones of identical reactive T cells called memory T cells. As noted above, most of the T cells in the body belong to one of two subsets and are distinguished by the presence on their surface of one or the other of two glycoproteins designated CD4 and CD8. Which of these molecules is present determines the types of cells to which the T cell can bind. T cells bearing CD8 ( $CD8^+$  T cells) always recognize antigen in association with Class I MHC proteins and typically function as cytotoxic T cells. Almost all the cells of the body express Class I MHC molecules. T cells bearing CD4 ( $CD4^+$  T cells) always recognize antigens in association with Class II MHC proteins on the surfaces of other cells. Only specialized antigen-presenting cells express Class II MHC molecules, including dendritic cells, phagocytic cells such as macrophages, and B cells.  $CD4^+$  T lymphocytes generally function as T helper cells.

T helper cells, which include Th1 cells and Th2 cells, respond to antigen with the production of lymphokines. Th1 and Th2 cells can be distinguished based on their lymphokine profiles. Like all T cells, Th cells arise in the thymus. When they are presented with an antigen by antigen-presenting dendritic cells they begin to proliferate and become

activated. There are two kinds of dendritic cell, DC1 cells (descended from monocytes) and DC2 cells (which appear to be derived from lymphocytes).

Th1 cells (inflammatory Th1 cells involved in the elimination of pathogens residing intracellularly in vesicular compartments) are produced when DC1-type dendritic cells present antigen to the T cell receptor for antigen (TCR) and secrete Interleukin 12 (IL-12). This paracrine stimulation activates Th1 cells to secrete their own lymphokines, in particular, Tumor-Necrosis Factor-beta (TNF- $\beta$ ) (also known as lymphotoxin) and Interferon-gamma (IFN- $\gamma$ ). These lymphokines stimulate macrophages to kill bacteria they have engulfed by phagocytosis and they recruit other leukocytes to the site producing inflammation. Th1 cells are essential for cell-mediated immunity and for controlling intracellular pathogens such as, for example, *Listeria* and *Mycobacterium tuberculosis*.

Th2 cells (“true” helper Th2 cells, which are required for antibody production by B cells) are produced when DC2-type dendritic cells present antigen to the T cell receptor for antigen and, presumably, one or more paracrine stimulants. The major lymphokines secreted by Th2 cells are Interleukin 4 (IL-4), which stimulates class-switching in B cells and promotes their synthesis of IgE antibodies, acts as a positive-feedback device promoting more pre-Th cells to enter the Th2 pathway, and blocks expression of the IL-12 receptor thereby inhibiting pre-Th cells in the thymus from entering the Th1 pathway. IL-4 also causes B cells to proliferate and differentiate into antibody-secreting plasma cells and memory B cells. IL-4 activates only B cells in the vicinity which themselves have bound the antigen, and not others, so as to sustain the specificity of the immune response. Th2 cells also produce Interleukin 5 (IL-5, which attracts and activates eosinophils), Interleukin 10 (IL-10, which inhibits IL-12 production by DCs and prevents maturation of pre-Th cells to Th1 cells), and Interleukin 13 (IL-13, which also promotes the synthesis of IgE antibodies).

Activation of the Th2 cell also causes it to begin to produce Interleukin 2 (IL-2), and to express a membrane receptor for IL-2. The secreted IL-2 autostimulates proliferation of Th2 cells. For example, IL-2 binds to IL-2 receptors on other T cells (which have bound the antigen) and stimulates their proliferation. In addition to IL-2, stimulated Th2 cells also produce IFN- $\gamma$  and Interleukin 6 (IL-6), which mediate various aspects of the immune response. IFN- $\gamma$  activates Natural Killer cells to their full cytolytic potential, and

is an activator of macrophages and thus increases their antitumor activities. If the macrophages are infected by intracellular parasites, it activates macrophages which in turn destroy the parasites. IFN- $\gamma$  also reinforces the antitumor activities of the cytotoxic lymphocytes, increases the nonspecific activities of NK-cells, and is one of the factors that controls the differentiation of B cells and increases the secretion of immunoglobins. IL-6 stimulates several types of leukocytes, as well as the production of Acute Phase Proteins in the liver. It is particularly important in inducing B cells to differentiate into antibody forming (plasma) cells. Thus, Th2 cells provide help for B cells and are essential for antibody-mediated immunity.

Cytotoxic T lymphocytes are able to kill cells that show a new or foreign antigen on their surface (for example, virus-infected cells, or tumor cells, or transplanted tissue cells). The CD8<sup>+</sup> CTLs also come in two subsets: Tc1 that, like Th1 cells, secrete IFN- $\gamma$ , and Tc2 that, like Th2 cells, secrete IL-4.

The cell-mediated immunity response also plays a role in destruction of tumor cells and in rejection of tissue transplants in animals. A major problem in tissue transplantation is rejection, which is often based on cell-mediated immunity response to “foreign” cells (because they are not a perfect antigenic match). Because tumor cells contain specific antigens not seen on normal cells they also may be recognized as foreign and destroyed by the forces of cell-mediated immunity. If tumor cells develop on a regular basis in animals, it may be cell-mediated immunity that eliminates them or holds them in check. The increase in the incidence of many types of cancer (tumors) in humans with advancement of age may be correlated with a decline in the peak efficiency of the immune system that occurs about 25 years of age.

A summary of the types of cells involved in the expression of cell-mediated immunity follows. Tc lymphocytes kill cells bearing foreign antigen on surface in association with Class I MHC and can kill cells that are harboring intracellular parasites (either bacteria or viruses) as long as the infected cell is displaying a microbial antigen on its surface. Tc cells kill tumor cells and account for rejection of transplanted cells. Tc cells recognize antigen-Class I MHC complexes on target cells, contact them, and release the contents of granules directly into the target cell membrane which lyses the cell. Th

lymphocytes produce lymphokines that are “helper” factors for development of B cells into antibody-secreting plasma cells. They also produce certain lymphokines that stimulate the differentiation of effector T lymphocytes and the activity of macrophages. Th1 cells recognize antigen on macrophages in association with Class II MHC and become activated  
5 (by IL-1) to produce lymphokines including IFN- $\gamma$  that activates macrophages and NK cells. These cells mediate various aspects of the cell-mediated immunity response including delayed-type hypersensitivity reactions. Th2 cells recognize antigen in association with Class II MHC on an APC and then produce interleukins and other substances that stimulate specific B cell and T cell proliferation and activity. Macrophages are important as antigen-  
10 presenting cells that initiate T cell interactions, development, and proliferation. Macrophages are also involved in expression of cell-mediated immunity because they become activated by IFN- $\gamma$  produced in a cell-mediated immunity response. Activated macrophages have increased phagocytic potential and release soluble substances that cause inflammation and destroy many bacteria and other cells. Natural Killer cells are cytotoxic  
15 cells that lyse cells bearing new antigen regardless of their MHC type and even lyse some cells that bear no MHC proteins. NK cells are defined by their ability to kill cells displaying a foreign antigen (*e.g.*, tumor cells) regardless of MHC type and regardless of previous sensitization (exposure) to the antigen. NK cells can be activated by IL-2 and IFN- $\gamma$ , and lyse cells in the same manner as cytotoxic T lymphocytes. Some NK cells have receptors  
20 for the Fc domain of the IgG antibody and are thus able to bind to the Fc portion of IgG on the surface of a target cell and release cytolytic components that kill the target cell via antibody-dependent cell-mediated cytotoxicity.

Extracellular factors that affect cell proliferation and differentiation have been defined as cytokines. These include the lymphokines, which are proteins produced by  
25 T-lymphocytes that have effects on the differentiation, proliferation, and activity of various cells involved in the expression of cell-mediated immunity. In general, lymphokines function by (1) focusing circulating leukocytes and lymphocytes into the site of immunological encounter; (2) stimulating the development and proliferation of B cells and T cells; (3) stimulating and preparing macrophages for their phagocytic tasks; (4) stimulating  
30 Natural Killer cells; and (5) providing antiviral cover and activity. A summary of various

important lymphokines follows. Initially referred to as lymphocyte activation factor, IL-1 is mainly a product of macrophages, and has a variety of effects on various types of cells. It acts as a growth regulator of T cells and B cells, and it induces other cells such as hepatocytes to produce proteins relevant to host defense. IL-1 forms a chemotactic gradient for neutrophils and serves as an endogenous pyrogen which produces fever. Thus, IL-1 plays an important role in both the immune responses and in the inflammatory response. IL-2 stimulates the proliferation of T cells and activates NK cells. IL-3 regulates the proliferation of stem cells and the differentiation of mast cells. IL-4 causes B cell proliferation and enhanced antibody synthesis. IL-6 (also referred to as Interferon-beta2, hybridoma growth factor, B-cell differentiation factor, and hepatocyte stimulatory factor) has effects on B cell differentiation and on antibody production and on T cell activation, growth, and differentiation, and probably has a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. IL-8 is a chemotactic attractant for neutrophils. IL-13 shares many of the properties of IL-4, and is a potent regulator of inflammatory and immune responses. IFN- $\gamma$  is produced by T cells and may be considered a lymphokine. It is sometimes called "immune interferon" (Interferon-alpha being referred to as "leukocyte interferon" and Interferon-beta being referred to as "fibroblast interferon"). IFN- $\gamma$  has several antiviral effects including inhibition of viral protein synthesis in infected cells. It also activates macrophages and NK cells, and stimulates IL-1, IL-2, and antibody production. Lymphotoxins include the Tumor Necrosis Factors. TNF-beta is produced by T cells, while TNF-alpha is produced by T cells as well as other types of cells. TNFs function to kill cells, including tumor cells (at a distance). There are several Colony Stimulating Factors (CSFs), including granulocyte macrophage colony stimulating factor (GM-CSF), which cause phagocytic white cells of all types to differentiate and divide.

The nature of the membrane receptors for antigen on B cells and T cells is fairly well understood. Each B cell has approximately  $10^5$  membrane-bound antibody molecules (IgD or IgM) that correspond in specificity to the antibody the cell is programmed to produce (these receptors being referred to as BCRs). CD32 (Fc $\gamma$ RII) on B cells are receptors for the Fc region of IgG. CD21 and CD35 on B cells are receptors for

complement components. Each T cell has about  $10^5$  molecules of a specific antigen-binding T cell receptor (a TCR) exposed on its surface. The TCR is similar, but not identical, to an antibody. There are two types of T cells that differ in their TCRs, alpha/beta ( $\alpha\beta$ ) T cells and gamma/delta ( $\gamma\delta$ ) T cells. The TCR of alpha/beta T cells binds a bimolecular complex displayed by a Class I MHC molecule at the surface of an antigen-presenting cell. As noted above, most Th cells express CD4, whereas most Tc cells express CD8.

Both BCRs and TCRs are similar in that they are integral membrane proteins, they are present in thousands of identical copies exposed at the cell surface, they are made before the cell ever encounters an antigen, they are encoded by genes assembled by the recombination of segments of DNA, they have a unique binding site that binds through non-covalent forces to a portion of the antigen called an epitope (or antigenic determinant) that depends on complementarity of the surface of the receptor and the surface of the epitope, and successful binding of the antigen receptor to the epitope, if accompanied by additional signals, results in stimulation of the cell to leave  $G_0$  and enter the cell cycle and repeated mitosis that leads to the development of a clone of cells bearing the same antigen receptor, *i.e.*, a clone of cells of the identical specificity. BCRs and TCRs differ in their structure, the genes that encode them, and the type of epitope to which they bind.

Induction of a primary immune response begins when an antigen penetrates epithelial surfaces. It will eventually come into contact with macrophages or certain other classes of antigen presenting cells, including B cells, monocytes, dendritic cells, Langerhans cells, and endothelial cells. Antigens, such as bacterial cells, are internalized by endocytosis and “processed” by APCs, then “presented” to immunocompetent lymphocytes to initiate the early steps of the immunological response. Processing by a macrophage (for example) results in attaching antigenic materials to the surface of the membrane in association with Class II MHC molecules on the surface of the cell. The antigen-class II MHC complex is presented to a T-helper (Th2) cell which is able to recognize processed antigen associated with a Class II MHC molecule on the membrane of the macrophage. This interaction, together with stimulation by IL-1 from secreted by the macrophage, will activate the Th2 cell.



As indicated above, B cells themselves behave as APCs. Cross-linked antigens bound to antibody receptors on the surface of a B cell cause internalization of some of the antigen and expression on the B cell membrane together with Class II MHC molecules. The Th2 cell recognizes the antigen together with the Class II MHC molecules, and secretes the various lymphokines that activate the B cells to become antibody-secreting plasma cells and memory B cells. Even if the antigen cannot cross-link the receptor, it may be endocytosed by the B cell, processed, and returned to the surface in association with Class II MHC where it can be recognized by specific Th2 cells which will become activated to initiate B cell differentiation and proliferation. In any case, the overall B cell response leads to antibody-mediated immunity.

The antigen receptors on B cell surfaces are thought to be the specific types of antibodies that they are genetically-programmed to produce. Hence, there are thousands of sub-populations of B cells distinguished by their ability to produce a unique antibody molecule. B cells can also react with a homologous antigen on the surface of the macrophage, or with soluble antigens. When a B cell is bound to antigen, and simultaneously is stimulated by IL-4 produced by a nearby Th2 cell, the B cell is stimulated to grow and divide to form a clone of identical B cells, each capable of producing identical antibody molecules. The activated B cells further differentiate into plasma cells which synthesize and secrete large amounts of antibody, and into memory B cells. The antibodies produced and secreted by the plasma cells will react specifically with the homologous antigen that induced their formation. Many of these reactions lead to host defense and to prevention of reinfection by pathogens. Memory cells play a role in secondary immune responses. Plasma cells are relatively short-lived (about one week) but produce large amounts of antibody during this period. Memory cells, on the other hand, are relatively long-lived and upon subsequent exposure to antigen they become quickly transformed into antibody-producing plasma cells.

Generation of cell mediated immunity begins when, for example, a cytotoxic T cell recognizes a processed antigen associated with Class I MHC on the membrane of a cell (usually an altered self cell, but possibly a transplanted tissue cell, or a eukaryotic parasite). Under stimulation by IL-2 produced by Th2 cells, the Tc cell becomes activated

to become a cytotoxic T lymphocyte capable of lysing the cell which is showing the new foreign antigen on its surface, a primary manifestation of cell-mediated immunity. The interaction between an antigen-presenting macrophage and a Th cell stimulates the macrophage to produce and secrete a Interleukin-1 that acts locally on the Th cell, stimulating the Th-cell to differentiate and produce its own cytokines (which may here be called lymphokines because they arise from a lymphocyte). These lymphokines have various functions. IL-4 has an immediate effect on nearby B cells. IL-2 has an immediate effect on T cells as described above.

Leucocytes also express adhesion promoting receptors that mediate cell-cell and cell-matrix interactions. These adhesive interactions are crucial to the regulation of haemopoiesis and thymocyte maturation, the direction and control of leucocyte traffic and migration through tissues, and the development of immune and non-immune inflammatory responses. Several families of adhesion receptors have been identified. The leucocyte integrin family comprises three alpha-beta heterodimeric membrane glycoproteins that share a common beta subunit, designated CD18. The alpha subunits of each of the three members, lymphocyte function associated antigen-1 (LFA-1), macrophage antigen-1 (Mac-1) and p150,95 are designated CD11a, b, and c respectively. These adhesion molecules play a critical part in the immune and inflammatory responses of leucocytes. The leucocyte integrin family is, in turn, part of the integrin superfamily, members of which are evolutionally, structurally and functionally related. Another Integrin subfamily found on leucocytes is the VLA group, so-called because the "very late activation antigens" VLA-1 and VLA-2 were originally found to appear late in T-cell activation. Members of this family function mainly as extracellular matrix adhesion receptors and are found both on haemopoietic and non-haemopoietic cells. They play a part in diverse cellular functions including tissue organisation, lymphocyte recirculation and T-cell immune responses. Another integrin subfamily, the cytoadhesins, are receptors on platelets and endothelial cells that bind extracellular matrix proteins. A second family of adhesion receptors is the immunoglobulin superfamily, members of which include CD2, LFA-3, and ICAM-1, which participate in T-cell adhesive interactions, and the antigen-specific receptors of T and B cells, CD4, CD8, and the MHC Class I and II molecules. Another recognized family of

adhesion receptors is the selectins, characterized by a common lectin domain. Leucocyte adhesion molecule-1 (LAM-1), which is the human homologue of the murine homing receptor, MEL-14, is expressed on leucocytes, while endothelial leucocyte adhesion molecule-1 (ELAM-1) and granule membrane protein (GMP-140) are expressed on  
5 stimulated endothelial cells and activated platelets.

Activation of an immune response requires physical cell-cell contact in addition to cytokines. Thus, for example, development of B and T cell precursors require intimate contact with stromal cells. At least three critical cell-cell contact events are required for the generation of immune responses. The first is initial contact of a specific  
10 antigen with a naive T cell. Because of the requirement for MHC presentation, this is an obligate cell contact event. In normal situations the critical antigen presenting cell is the dendritic cell. In addition to the MHC/peptide-TCR interaction there are other non-antigen specific membrane bound ligand-receptor pairs which are important for the dendritic cell-T cell interaction. The principal one is the association of the CD28 molecule on the T cell  
15 with either of two ligands, B7.1 (CD80) and B7.2 (CD86), on the dendritic cell. These molecules are termed accessory molecules and it is understood that the CD28 molecule delivers an essential second signal to the T cell without which the T cell does not become activated.

A second essential cell-cell contact is between the activated T cell and an  
20 antigen-specific B cell. Most antigens are T cell-dependent, that is, an antibody response to the antigen absolutely requires T cell help. This help is delivered both by cytokines and by cell-cell contact. Cells bind specific antigen via surface Ig, then internalize, process, and present it on Class II MHC molecules. This enables them to be recognized by T cells specific for helper epitopes from the specific antigen. This cell-cell interaction also requires  
25 CD28 binding to B7 on the B cell. In addition, a molecule called CD40 ligand or CD154, the expression of which is induced upon T cell activation, binds to CD40 on B cells. CD40 crosslinking promotes B cell proliferation, prevents apoptosis of germinal-center B cells, and promotes immunoglobulin isotype switching. The CD28-B7 and CD40-CD40L receptor ligand interactions are both essential for the dialogue between B and T cells that  
30 causes their mutual activation.

A third cell-cell interaction that is essential in immune responses is the binding of activated B cells (which have migrated into a specialised structure in lymphoid organs called germinal centers) to follicular dendritic cells (FDCs). FDCs are specialized stromal cells that hold intact, *i.e.*, unprocessed, antigen on their surface in the form of long-lived immune complexes. Among other molecules, FDCs express CD23, which binds to germinal center B cells via a CR2 receptor and stimulates differentiation to plasma cells. Time is required before a primary immune response is effective as a host defense. Antigens have to be recognized, taken up, digested, processed, and presented by APCs. A few select Th cells must react with antigen and respond; preexisting B or T lymphocytes must encounter the antigen and proliferate and differentiate into effector cells (plasma cells or Tc cells). In the case of antibody-mediated immunity, antibody level has to build up to an effective physiological concentration to render its host resistant. It may take several days or weeks to reach a level of effective immunity, even though this immunity may persist for many months, or years, or even a lifetime, due to the presence of the antibodies. In natural infections, the inoculum is small, and even though the antigenic stimulus increases during microbial replication, only small amounts of antibody are formed within the first few days, and circulating antibody is not detectable until about a week after infection.

With regard to induction of a secondary immune response, it is understood that on re-exposure to microbial antigens (secondary exposure to antigen), there is an accelerated immunological response, *i.e.*, the secondary or memory response. Larger amounts of antibodies are formed in only 1-2 days. This is due to the activities of specific memory B cells or memory T cells which were formed during the primary immune response. These memory cells, when stimulated by homologous antigen, “remember” having previously seen the antigen, and are able to rapidly divide and differentiate into effector cells. Stimulating memory cells to rapidly produce very high (effective) levels of persistent circulating antibodies is the basis for giving “booster”-type vaccinations to humans and pets. Thus, following the first exposure to an antigen the immune response (as evidenced by following the concentration of specific antibody in the serum) develops gradually over a period of days, reaches a low plateau within 2-3 weeks, and usually begins to decline in a relatively short period of time. When the antigen is encountered a second

time, a secondary (memory) response causes a rapid rise in the concentration of antibody, reaching a much higher level in the serum, which may persist for a relatively long period of time. This is not to say that a protective level of antibody may not be reached by primary exposure alone, but usually to ensure a high level of protective antibody that persists over a  
5 long period of time, it is necessary to have repeated antigenic stimulation of the immune system.

An immunoglobulin molecule (abbreviated Ig), is a multimeric protein composed of two identical light chain polypeptides and two identical heavy chain polypeptides ( $H_2L_2$ ) that are joined into a macromolecular complex by interchain disulfide  
10 bonds, *i.e.*, covalent bonds between the sulfhydryl groups of neighboring cysteine residues. There are various classes of human antibody proteins, each of which is produced by a specific clone of plasma cells. Five human immunoglobulin classes are defined on the basis of their heavy chain composition, and are named IgG, IgM, IgA, IgE, and IgD. The IgG-class and IgA-class antibodies are further divided into subclasses, namely, IgG1, IgG2,  
15 IgG3, and IgG4, and IgA1 and IgA2. Intrachain disulfide bonds join different areas of the same polypeptide chain, which results in the formation of loops that, along with adjacent amino acids, constitute the immunoglobulin domains. At the amino-terminal portion (also called the "NH<sub>2</sub>-terminus" or the "N-terminus"), each light chain and each heavy chain has a single variable region that shows considerable variation in amino acid composition from one  
20 antibody to another. The light chain variable region,  $V_L$ , associates with the variable region of a heavy chain,  $V_H$ , to form the antigen binding site of the immunoglobulin, called the Fv.

In addition to variable regions, each of the antibody chains have one or more constant regions. Light chains have a single constant region domain. Thus, light chains have one variable region and one constant region. Heavy chains have several constant  
25 region domains. The heavy chains in IgG, IgA, and IgD antibodies have three constant region domains, which are designated CH1, CH2, and CH3, and the heavy chains in IgM and IgE antibodies have four constant region domains, CH1, CH2, CH3 and CH4. Thus, heavy chains have one variable region and three or four constant regions. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., *Antibodies: A*

*Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988).

The heavy chains of immunoglobulins can also be divided into three functional regions: the Fd region (a fragment comprising V<sub>H</sub> and CH1, *i.e.*, the two N-terminal domains of the heavy chain), the hinge region, and the Fc region (the "fragment crystallizable" region, derived from constant regions and formed after pepsin digestion). The Fd region in combination with the light chain forms an Fab (the "fragment antigen-binding"). Because an antigen will react stereochemically with the antigen-binding region at the amino terminus of each Fab the IgG molecule is divalent, *i.e.*, it can bind to two antigen molecules. The Fc contains the domains that interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Thus, the Fc fragment is generally considered responsible for the effector functions of an immunoglobulin, such as complement fixation and binding to Fc receptors. Pepsin sometimes also cleaves before the third constant domain (CH3) of the heavy chain to give a large fragment F(abc) and a small fragment pFcb. These terms are also used for analogous regions of the other immunoglobulins. The hinge region, found in IgG, IgA, and IgD class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses.

For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, it is relatively short and contains a rigid poly-proline double helix, stabilised by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3 the Fab fragments are relatively far away from the Fc fragment,

giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge region reportedly decreases in the order  
5 IgG3>IgG1>IgG4>IgG2. The four IgG subclasses also differ from each other with respect to their effector functions. This difference is related to differences in structure, including with respect to the interaction between the variable region, Fab fragments, and the constant Fc fragment. Nevertheless, aside from glycosylation within the CH2 region, for example, in spite of this knowledge there are no set rules or conventions regarding means or methods to  
10 change features, including sequences, of these subclasses of molecule to change, control, add, or remove different functions, for example, ADCC, CDC, and other functions.

According to crystallographic studies, the immunoglobulin hinge region can be further subdivided functionally into three regions: the upper hinge region, the core region, and the lower hinge region. Shin *et al.*, 1992 *Immunological Reviews* 130:87. The upper  
15 hinge region includes amino acids from the carboxyl end of CH1 to the first residue in the hinge that restricts motion, generally the first cysteine residue that forms an interchain disulfide bond between the two heavy chains. The length of the upper hinge region correlates with the segmental flexibility of the antibody. The core hinge region contains the inter-heavy chain disulfide bridges, and the lower hinge region joins the amino terminal end  
20 of the CH2 domain and includes residues in CH2. *Id.* The core hinge region of human IgG1 contains the sequence Cys-Pro-Pro-Cys which, when dimerized by disulfide bond formation, results in a cyclic octapeptide believed to act as a pivot, thus conferring flexibility. The hinge region may also contain one or more glycosylation sites, which include a number of structurally distinct types of sites for carbohydrate attachment. For example, IgA1 contains  
25 five glycosylation sites within a 17 amino acid segment of the hinge region, conferring resistance of the hinge region polypeptide to intestinal proteases, considered an advantageous property for a secretory immunoglobulin.

Conformational changes permitted by the structure and flexibility of the immunoglobulin hinge region polypeptide sequence may also affect the effector functions of  
30 the Fc portion of the antibody. Three general categories of effector functions associated

with the Fc region include (1) activation of the classical complement cascade, (2) interaction with effector cells, and (3) compartmentalization of immunoglobulins. The different human IgG subclasses vary in the relative efficacies with which they fix complement, or activate and amplify the steps of the complement cascade. See, e.g., Kirschfink, 2001 *Immunol. Rev.* **180**:177; Chakraborti *et al.*, 2000 *Cell Signal* **12**:607; Kohl *et al.*, 1999 *Mol. Immunol.* **36**:893; Marsh *et al.*, 1999 *Curr. Opin. Nephrol. Hypertens.* **8**:557; Speth *et al.*, 1999 *Wien Klin. Wochenschr.* **111**:378.

Complement-dependent cytotoxicity (CDC) is believed to be a significant mechanism for clearance of specific target cells such as tumor cells. CDC is a stream of events that consists of a series of enzymes that become activated by each other in a cascade fashion. Complement has an important role in clearing antigen, accomplished by its four major functions: (1) local vasodilation; (2) attraction of immune cells, especially phagocytes (chemotaxis); (3) tagging of foreign organisms for phagocytosis (opsonization); and (4) destruction of invading organisms by the membrane attack complex (MAC attack). The central molecule is the C3 protein. It is an enzyme that is split into two fragments by components of either the classical pathway or the alternative pathway. The classical pathway is induced by antibodies, especially IgG and IgM, while the alternative pathway is nonspecifically stimulated by bacterial products like lipopolysaccharide (LPS). Briefly, the products of the C3 split include a small peptide C3a which is chemotactic for phagocytic immune cells and results in local vasodilation by causing the release of C5a fragment from C5. The other part of C3, C3b coats antigens on the surface of foreign organisms and acts to opsonize the organism for destruction. C3b also reacts with other components of the complement system to form an MAC consisting of C5b, C6, C7, C8 and C9.

In general, IgG1 and IgG3 most effectively fix complement, IgG2 is less effective, and IgG4 does not activate complement. Complement activation is initiated by binding of C1q, a subunit of the first component C1 in the cascade, to an antigen-antibody complex. Even though the binding site for C1q is located in the CH2 domain of the antibody, the hinge region influences the ability of the antibody to activate the cascade. For example, recombinant immunoglobulins lacking a hinge region are unable to activate complement. Shin *et al.*, 1992. Without the flexibility conferred by the hinge region, the



Fab portion of the antibody bound to the antigen may not be able to adopt the conformation required to permit C1q to bind to CH2. *See id.* Hinge length and segmental flexibility have been reported to correlate with complement activation; however, the correlation is not absolute. Human IgG3 molecules with altered hinge regions that are as rigid as IgG4, for example, can still effectively activate the cascade.

The absence of a hinge region, or a lack of a functional hinge region, can also affect the ability of certain human IgG immunoglobulins to bind Fc receptors on immune effector cells. Binding of an immunoglobulin to an Fc receptor facilitates antibody-dependent cell-mediated cytotoxicity, which as noted above is presumed to be an important mechanism for the elimination of tumor cells. The human IgG Fc receptor (FcR) family is divided into three groups, FcγRI (CD64), which is capable of binding IgG with high affinity, and FcγRII (CD32) and FcγRIII (CD16), both of which are lower affinity receptors. The molecular interaction between each of the three receptors and an immunoglobulin has not been defined precisely, but experimental evidence indicates that residues in the hinge proximal region of the CH2 domain may be important to the specificity of the interaction between the antibody and the Fc receptor. IgG1 myeloma proteins and recombinant IgG3 chimeric antibodies that lack a hinge region are reportedly unable to bind FcγRI, perhaps because accessibility to CH2 is decreased. Shin *et al.*, 1993 *Intern. Rev. Immunol.* 10:177, 178-79.

Unusual and apparently evolutionarily unrelated exceptions to the H<sub>2</sub>L<sub>2</sub> structure of conventional antibodies occur in some isotypes of the immunoglobulins found in camelids (camels, dromedaries and llamas; Hamers-Casterman *et al.*, 1993 *Nature* 363:446; Nguyen *et al.*, 1998 *J. Mol. Biol.* 275:413), nurse sharks (Roux *et al.*, 1998 *Proc. Nat. Acad. Sci. USA* 95:11804), and in the spotted ratfish (Nguyen, *et al.*, "Heavy-chain antibodies in Camelidae; a case of evolutionary innovation," 2002 *Immunogenetics* 54(1):39-47). These antibodies can apparently form antigen-binding regions using only heavy chain variable region, *i.e.*, these functional antibodies are homodimers of heavy chains only (referred to as "heavy-chain antibodies" or "HCAbs"). In both species, these variable regions often contain an extended third complementarity determining region (CDR3) that may help compensate for the lack of a light chain variable region, and there are

frequent disulfide bonds between CDR regions that presumably help to stabilize the binding site. Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129; Roux *et al.*, 1998. However, the precise function of the heavy chain-only “antibodies” is unknown, and the evolutionary pressure leading to their formation has not been identified. See, *e.g.*, Nguyen, *et al.*, 2002, *supra*. Camelids, including camels, llamas, and alpacas, also express conventional H<sub>2</sub>L<sub>2</sub> antibodies, and the heavy chain-only antibodies thus do not appear to be present in these animals simply as an alternative antibody structure.

Variable regions (V<sub>H</sub>H) of the camelid heavy chain-only immunoglobulins and conventional (H<sub>2</sub>L<sub>2</sub>) heavy chain variable regions contain amino acid differences, including differences at several positions that may be involved in the interface between conventional V<sub>H</sub> and V<sub>L</sub> domains. Nguyen *et al.*, 1998 *J. Mol. Biol* 275:413; Muyldermans *et al.*, 1994 *Prot. Engineer.* 7:1129. Camelid V<sub>H</sub>H reportedly recombines with IgG2 and IgG3 constant regions that contain hinge, CH2, and CH3 domains and lack a CH1 domain. Hamers-Casterman *et al.*, 1993 *Nature* 363:446. Interestingly, V<sub>H</sub>H are encoded by a chromosomal locus distinct from the V<sub>H</sub> locus (Nguyen *et al.*, 1998, *supra*), indicating that camelid B cells have evolved complex mechanisms of antigen recognition and differentiation. Thus, for example, llama IgG1 is a conventional (H<sub>2</sub>L<sub>2</sub>) antibody isotype in which V<sub>H</sub> recombines with a constant region that contains hinge, CH1, CH2 and CH3 domains, whereas the llama IgG2 and IgG3 are heavy chain-only isotypes that lack CH1 domains and that contain no light chains.

The classes of immunoglobulins have different physical and chemical characteristics and they exhibit unique biological properties. Their synthesis occurs at different stages and rates during an immune response and/or during the course of an infection. Their importance and functions in host resistance (immunity) are different.

Immunoglobulin G (IgG), a protein with a molecular weight of about 150,000 daltons (150kD), is the predominant Ig in the serum. It makes up about 80% of the total antibody found in an animal at any given time, being 75% of the total serum antibody. It can diffuse out of the blood stream into the extravascular spaces and it is the most common Ig found there. Its concentration in tissue fluids is increased during inflammation, and it is particularly effective at the neutralization of bacterial extracellular toxins and viruses. It

also has opsonizing ability and complement-fixing ability. The polypeptide composition of the Fc region of all IgG1 antibody molecules is relatively constant regardless of antibody specificity; however, as noted above, the Fab regions always differ in their exact amino acid sequences depending upon their antigenic specificity. Specific amino acid regions of the Fc portion of the molecule are recognized by receptors on phagocytes and certain other cells, and the Fc domain contains a peptide region that will bind to and activate complement, which is often required for the manifestation of antibody-mediated immunity. Because the IgG molecule is divalent, it can cross-link antigen molecules, which may lead to precipitation or agglutination of antigens; if IgG is bound to antigen on a microbial cell or surface, its Fc region may provide an extrinsic ligand that will be recognized by specific receptors on phagocytes. Microbial cells or viruses coated with IgG molecules are opsonized for phagocytosis, and opsonized pathogens are taken up and destroyed much more readily by phagocytes than their non-opsonized counterparts. IgG, as well as IgM and IgA, will neutralize the activity of toxins, including bacterial exotoxins. Furthermore, cross-linked IgG molecules on the surface of a cell can bind and activate complement from the serum and set off a cascade of reactions that can lead to destruction of the cell.

IgM is the first immunoglobulin to appear in the blood stream during the course of an infection. It is mainly confined to the bloodstream and provides protection against blood-borne pathogens. IgM makes up about 10% serum immunoglobulins, and is arranged to resemble a pentamer of five immunoglobulin molecules (having a molecular weight of about 900kD) tethered together at by their Fc domains. In addition to covalent linkages between the monomeric subunits, the pentamer is stabilized by a 15kd polypeptide called J chain. IgM, therefore, has a theoretical “valence” of ten (*i.e.*, it has ten exposed Fab domains). Probably, the most important role of IgM is its ability to function early in the immune responses against blood-borne pathogens given its efficiency in agglutinating particulate antigens. IgM binds also complement strongly and IgM antibodies bound to a microbial surface act as opsonins, rendering the microbe more susceptible to phagocytosis. In the presence of complement and IgM whole microbial cells may be killed and lysed. As noted above, IgM also appears on the surfaces of mature B cells as a transmembranous

monomer where it functions as an antigen receptor, capable of activating B cells when bound to antigen.

Gene rearrangement at the immunoglobulin loci during lymphoid development generates a repertoire of B lymphocytes that express a diversity of antigen receptors. The gene rearrangement, which is catalysed by the rearrangement-activating gene ("RAG") recombinase, integrates the immunoglobulin V, D and J gene segments to yield productively rearranged immunoglobulin genes that encode the heavy and light chains of IgM antibodies. The diversity of IgM antibodies in this primary repertoire is achieved through combinatorial mechanisms (the choice of V, D and J gene segments utilized in a particular antibody), as well as junctional diversity. The joining of V, D and J gene segments is somewhat imprecise, and nucleotides may be inserted at the junction in a non-templated manner. There is therefore a very high degree of resultant diversity at the V-D-J borders. This contributes in a major way to the structural diversity of the third complementarity determining region of the antibody, a region that often plays a critical role in antigen recognition. This primary repertoire of IgM antibodies comprises a few million different structures. The size of this repertoire means that any incoming antigen is likely to encounter an antibody that recognizes it with acceptable affinity. A high-affinity binding site is unlikely to be available for most incoming antigens (the repertoire is not large enough), but the affinity of the available IgM antibodies in the primary repertoire will vary from antigen to antigen. If an epitope is re-iterated at high density on the surface of the antigen (*e.g.*, a repeated structure on the surface of a virus or bacterium), then an IgM antibody may nevertheless be effective in mediating clearance of the organism, despite the low affinity of the individual interaction between antigenic epitope and immunoglobulin combining site. The density of the epitopes may allow multivalent interactions with IgM, leading to a high-avidity interaction, providing that a suitable spacing of antigenic epitopes can occur. Nevertheless, to ensure an effective and specific response, especially when the concentration of antigen is low (as may occur when the body is faced with a very small number of infecting viral particles), it would be preferable if high-affinity antibodies were available for neutralizing, for example, an infecting organism. The size of the primary repertoire mitigates against the likelihood of such high-affinity antibodies being present in

this repertoire. The immune system therefore operates using a two-stage strategy. The primary repertoire of IgM antibodies is generated by a process of gene rearrangement and takes place prior to antigen encounter during early lymphocyte development. However, once foreign antigen has been encountered, those B cells in the primary repertoire that  
5 encode suitable (albeit low-affinity) antibodies are selectively expanded and subjected to an iterative alternation of directed hypermutation and antigen-mediated selection. This allows a significant maturation in affinity of the antigen-specific antibodies that are produced. Antigen triggering also drives isotype switch recombination. Thus, in the absence of external antigen stimulation and any maternally derived immunoglobulin, the serum will  
10 only contain a diversity of unmutated IgM molecules that have been generated by gene rearrangement. This repertoire shifts with age as a result of continuous antigen exposure, such that the majority of the serum immunoglobulin in older animals is composed of mutated IgG (and IgA) molecules whose specificities have developed as a consequence of antigen selection.

15 IgA exists as a  $H_2L_2$  monomer of about 160kD in serum and, in secretions, as a dimer of the  $H_2L_2$  monomer of about 400kD. As with IgM, polymerization (dimerization) is via a J-chain. IgA has two subclasses based on different heavy chains, IgA1 and IgA2. IgA1 is produced in bone marrow and makes up most of the serum IgA. Both IgA1 and IgA2 are synthesized in GALT (gut associated lymphoid tissues) to be secreted onto the  
20 mucosal surfaces. Because IgA may be synthesized locally and secreted in the seromucous secretions of the body, it is sometimes referred to as secretory antibody or sIgA. Quantitatively, IgA is synthesized in amounts greater than IgG, but it has a short half life in serum (6 days), and it is lost in secretory products. The concentration of IgA in serum is about 15% of the total antibody. Secretion of dimeric IgA is mediated by a 100kD  
25 glycoprotein called secretory component. It is the addition of the secretory piece to IgA molecules that accounts for their ability to exit the body to mucosal surfaces via the exocrine glands. IgM can be transported similarly and makes up a small proportion of secretory antibodies. Secretory IgA is the predominant immunoglobulin present in gastrointestinal fluids, nasal secretions, saliva, tears and other mucous secretions of the body. IgA  
30 antibodies are important in resistance to infection of the mucosal surfaces of the body,

particularly the respiratory, intestinal and urogenital tracts. IgA acts as a protective coating for the mucous surfaces against microbial adherence or initial colonization. It can also neutralize toxin activity on mucosal surfaces. Fc receptors for IgA-coated microorganisms found on monocytes and neutrophils derived from the respiratory mucosa suggest that IgA  
5 may have a role in the lung, at least, in opsonization of pathogens. Secretory IgA is also transferred via the milk, *i.e.*, the colostrum, from a nursing mother to a newborn, which provides passive immunity to many pathogens, especially those that enter by way of the GI tract.

IgE is a immunoglobulin of about 190kD that accounts for about 0.002% of  
10 the total serum immunoglobulins. It is produced by plasma cells below the respiratory and intestinal epithelia. The majority of IgE is bound to tissue cells, especially mast cells. If an infectious agent succeeds in penetrating the IgA barrier, it comes up against the next line of defense, the MALT (mucosa-associated lymphoid tissues) system which is managed by IgE. IgE is bound very firmly to specific IgE Fc receptors on mast cells. Contact with antigen  
15 leads to release of mediators of inflammation from the mast cells, which effectively recruits various agents of the immune response including complement, chemotactic factors for phagocytes, T cells, *etc.* Although a well-known manifestation of this reaction is a type of immediate hypersensitivity reaction called atopic allergy (*e.g.*, hives, asthma, hay fever, *etc.*), the MALT responses act as a defense mechanism because they amplify the  
20 inflammatory response and may facilitate rejection of a pathogen.

IgD is a molecule of about 175kd that resembles IgG in its monomeric form. IgD antibodies are found for the most part on the surfaces of B lymphocytes. The same cells may also carry IgM antibody. As noted above, it is thought that IgD and IgM function as mutually-interacting antigen receptors for control of B cell activation and suppression.  
25 Hence, IgD may have an immunoregulatory function.

In addition to opsonization, activation of complement, and ADCC, antibodies have other functions in host defense including steric hindrance, toxin neutralization, agglutination, and precipitation. With regard to steric hindrance, it is understood that antibodies combine with the surfaces of microorganisms and may block or prevent their  
30 attachment to susceptible cells or mucosal surfaces. Antibody against a viral component can

block attachment of the virus to susceptible host cells and thereby reduce infectivity. Secretory IgA can block attachment of pathogens to mucosal surfaces. Toxin-neutralizing antibodies (antitoxins) can also react with a soluble bacterial toxin and block the interaction of the toxin with its specific target cell or substrate. Antibodies can also combine with the surfaces of microorganisms or soluble antigens and cause them to agglutinate or precipitate. This reduces the number of separate infectious units and makes them more readily phagocytosed because the clump of particles is larger in size. Floccules or aggregates of neutralized toxin may be removed by phagocytes.

Antibodies have been proposed for use in therapy. Animals, including humans and mice have the ability to make antibodies able to recognize (by binding to) virtually any antigenic determinant and to discriminate between similar epitopes. Not only does this provide the basis for protection against disease organisms, but it makes antibodies attractive candidates to target other types of molecules found in the body, such as receptors or other proteins present on the surface of normal cells and molecules present uniquely on the surface of cancer cells. Thus the remarkable specificity of antibodies makes them promising agents for human therapy.

Initial antibody preparations available for use, such as intravenous gammaglobulins, included animal and human antisera that were used *in vivo* to destroy bacteria (tetanus, pneumococcus) and neutralize virus (hepatitis A and B, rabies, cytomegalovirus, and varicella zoster) in the blood of infected individuals. Possibly the most important early application was the use and endotoxins. However, there are problems associated with the use of antibodies in human therapy because the response of the immune system to any antigen, even the simplest, is "polyclonal," *i.e.*, the system manufactures antibodies of a great range of structures both in their binding regions as well as in their effector regions. Polyclonal antibody treatment was also associated with unwanted side effects. In addition to the polyclonal nature of these antibody preparations, there was the risk of infection from contaminating viruses. Serum sickness, anaphylaxis, and were also considered limiting factors. Furthermore, even if one were to isolate a single antibody-secreting cell, and place it in culture, it would die out after a few generations because of the limited growth potential of all normal somatic cells.

Until the late 1970s, polyclonal antibodies obtained from the blood serum of immunized animals, provided the only source of antibodies for research or treatment of disease. Isolation of specific antibodies was essentially impossible until Kohler and Milstein discovered how to make "monoclonal antibodies" that would have a single specificity, that  
5 would all be alike due to manufacture by a single clone of plasma cells, and that could be grown indefinitely. This technique was described in a 1975 publication (*Nature* 256:495-97), and Köhler and Milstein received the 1984 Nobel Prize in Medicine for their work.

The first step in Kohler and Milstein's technique for production of monoclonal antibodies involves immunizing an experimental animal with the antigen of  
10 interest. In most of their experiments, Kohler and Milstein injected a mouse with sheep red blood cells. The mouse's body initiates an immune response and begins producing antibodies specific to the antigen. The mouse's spleen is then removed and B cells producing the antibody of interest are isolated. Tumor-producing cells that have been grown in culture are then fused with the B lymphocytes using polyethylene glycol in order to  
15 produce a "hybridoma." Only hybridomas resulting from the fusion will survive. The spleen lymphocyte has a limited life span, so any B cells that did not fuse with a myeloma will die in the culture. As well, those cells that lack the antibody-producing aspect of the B cell will not secrete the enzyme HGPRT, which is required for growth in the HAT medium. The hypoxanthine-aminopterin-thymidine (HAT) medium, on which the cells are grown,  
20 inhibits the pathway for nucleotide synthesis. Cells which produce HGPRT can bypass this pathway and continue to grow. By placing the fused cells in a HAT medium, the true hybridomas can be isolated (McKay, Raff, Reichardt 1981). The isolated hybridoma cells are then screened for specificity to the desired antigen. Because each hybridoma descends from one B cell, it makes copies of only one antibody. The hybridoma that produces the  
25 antibody of interest is grown in culture to produce large amounts of monoclonal antibodies, which are then isolated for further use. The technique is called somatic cell hybridization, and the resulting hybridoma (selected for both immortality and production of the specific antibody of interest) may be cultured indefinitely, *i.e.*, it is a potentially immortal cell line.

Monoclonal antibodies are now widely used as diagnostic and research  
30 reagents. However, their introduction into human therapy has been much slower. One



principal difficulty is that mouse antibodies are “seen” by the human immune system as foreign, and the human patient mounts an immune response against them, producing HAMA (“human anti-mouse antibodies”). These not only cause the therapeutic antibodies to be quickly eliminated from the host, but also form immune complexes that cause damage to the kidneys.

Two approaches have been used in an attempt to reduce the problem of HAMA. The first is the production of chimeric antibodies in which the antigen-binding part (variable regions) of a mouse monoclonal antibody is fused to the effector part (constant region) of a human antibody using genetic engineering. In a second approach, rodent antibodies have been altered through a technique known as complementarity determining region (CDR) grafting or “humanization.” In this process, the antigen binding sites, which are formed by three CDRs of the heavy chain and three CDRs of the light chain, are excised from cells secreting rodent mAb and grafted into the DNA coding for the framework of the human antibody. Because only the antigen-binding site CDRs, rather than the entire variable domain of the rodent antibody are transplanted, the resulting humanized antibody (a second generation or “hyperchimeric” antibody) is reportedly less immunogenic than a first generation chimeric antibody. This process has been further improved to include changes referred to as “reshaping” (Verhoeven, *et al.*, “Reshaping human antibodies: grafting an anti-lysozyme activity,” 1988 *Science* **239**:1534-1536; Riechmann, *et al.*, “Reshaping human antibodies for therapy,” 1988 *Nature* **332**:323-337; Tempest, *et al.*, “Reshaping human monoclonal antibody to inhibit respiratory syncytial virus infection in vivo,” *Bio/Technol* 1991 **9**:266-271), “hyperchimerization” (Queen, *et al.*, “A humanized antibody that binds to the human interleukin 2 receptor,” 1989 *Proc Natl Acad Sci USA* **86**:10029-10033; Co, *et al.*, “Humanized antibodies for antiviral therapy,” 1991 *Proc Natl Acad Sci USA* **88**:2869–2873; Co, *et al.*, “Chimeric and humanized antibodies with specificity for the CD33 antigen,” 1992 *J Immunol* **148**:1149-1154), and “veneering” (Mark, *et al.*, “Derivation of therapeutically active humanized and veneered anti-CD18 antibodies. In: Metcalf BW, Dalton BJ, eds. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994:291-312). In the reshaping process on the basis of homology, the rodent variable region is compared with the consensus sequence of the protein sequence subgroup to which

it belongs. Similarly, the selected human constant region accepting framework is compared with its family consensus sequence. Gussowal, *et al.*, "Humanization of monoclonal antibodies," 1991 *Meth Enzymol* 203:99-121. The sequence analyses identify residues, which may have undergone mutation during the affinity maturation procedure and may therefore be idiosyncratic. Inclusion of the more common human residues is said to lessen immunogenicity problems by replacing human acceptor idiosyncratic residues. Further, the reshaping process is said to allow comparison of human and rodent consensus sequences to identify any systematic "species" differences. RSV19 antibodies were humanized by employing this procedure. Taylor *et al.*, "Humanized monoclonal antibody to respiratory syncytial virus," 1991 *Lancet* 337:1411-1412; Tempest, *et al.*, "Reshaping a human monoclonal antibody to inhibit human respiratory syncytial virus infection in vivo," 1991 *Bio/Technol* 9:266-271. Hyperchimerization is an alternative method of identifying residues outside CDR regions that are likely to be involved in the reconstitution of binding activity. In this method, the human sequences are compared with murine variable region sequences and the one with highest homology is selected as the acceptor framework. As in the reshaping procedure, the "idiosyncratic" residues are replaced by more commonly occurring human residues. The non-CDR residues that may be interacting with the CDR sequences are identified. Finally, it is determined which one of these residues is to be included in the variable region framework. Humanized antibodies against CD33 antigen were reportedly developed by this method. Co, *et al.*, "Chimeric and humanized antibodies with specificity for the CD33 antigen," 1992 *J Immunol* 148:1149-154. See also Carter, *et al.*, "Humanization of an anti-p185 HER2 antibody for human cancer therapy," 1992 *Proc Natl Acad Sci USA* 89:4285-4289. The displayed surface of the protein is the primary determinant of its immunogenicity. A humanized murine antibody can thus be made less immunogenic by replacing exposed residues that differ from those commonly found in human antibodies. This method of humanization is referred to as "veneering." Appropriate replacement of the outer residues may have little or no impact on the inner domains or interdomain framework.. Veneering is a two-step process. In the first step, the most homologous human variable regions are selected and compared by each single residue to the corresponding mouse variable regions. In the second step, the mouse framework residues,

which differ from its human homologue, are replaced by the residues present in the human homologue. This replacement involves only those residues that are on the surface and at least partially exposed.

Nevertheless, it took more than a quarter century of research for monoclonal antibody technology and genetic engineering methods to result in the development of immunoglobulin molecules for treatment of human diseases. Indeed, it was not until the past five years that monoclonal antibodies became as an expanding class of therapeutics. See Glennie MJ and van de Winkel JG, *Drug Discov Today* 2003 Jun 1;8(11):503-10; Souriau C and Hudson PJ, "Recombinant antibodies for cancer diagnosis and therapy," 2003 *Expert Opin Biol Ther.* 3(2):305-18. See also Pendley C, *et al.*, "Immunogenicity of therapeutic monoclonal antibodies," 2003 *Curr Opin Mol Ther.* 5(2):172-9.

All the same, an average of less than one therapeutic antibody per year has been introduced to the market beginning in 1986, eleven years after the publication of monoclonal antibodies. Five murine monoclonal antibodies were introduced into human medicine over a ten year period from 1986-1995, including "muromonab-CD3" (OrthoClone OKT3®), which binds to a molecule on the surface of T cells and was launched in 1986 to prevent acute rejection of organ transplants; "edrecolomab" (Panorex®), launched in 1995 for treatment of colorectal cancer; "odulimomab" (Antilfa®), launched in 1997 for use in transplant rejection; and, "ibritumomab" (Zevalin® yiuixetan), launched in 2002 for use in non-Hodgkin's lymphoma. Additionally, one monoclonal Fab, "abciximab" (ReoPro®), was launched in 1995. It inhibits the clumping of platelets by binding the receptors on their surface that normally are linked by fibrinogen and may be helpful in preventing reclogging of the coronary arteries in patients who have undergone angioplasty. Three chimeric monoclonal antibodies were also launched: "rituximab" (Rituxan®), in 1997, which binds to the CD20 molecule found on most B cells and is used to treat B cell lymphomas; "basiliximab" (Simulect®), in 1998 for transplant rejection; and "infliximab" (Remicade®) which binds to tumor necrosis factor-alpha (TNF-a), in 1998 for treatment of as rheumatoid arthritis and Crohn's disease. Additionally, "abciximab" (ReoPro®), a 47.6 kD Fab fragment of the chimeric human-murine monoclonal antibody 7E3 that binds to the glycoprotein (GP) IIb/IIIa receptor of human platelets, was launched in 1995 as an adjunct

to percutaneous coronary intervention for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention. Finally, seven “humanized” monoclonals were launched from 1997-2003: “daclizumab” (Zenapax®) in 1997, which binds to part of the IL-2 receptor produced at the surface of activated T cells and is used to prevent acute rejection of transplanted kidneys; “palivizumab” (Synagis®) in 1998 for RSV; “trastuzumab” (Herceptin®) in 1998, which binds HER-2, a growth factor receptor found on breast cancers cells; “gemtuzumab” (Mylotarg®) in 2000, which is a conjugate of a monoclonal antibody that binds CD33, a cell-surface molecule expressed by the cancerous cells in acute myelogenous leukemia (AML) but not found on the normal stem cells needed to repopulate the bone marrow; and “alemtuzumab” (MabCampath®) in 2001, which binds to CD52, a molecule found on white blood cells and has produced temporary remission of chronic lymphocytic leukemia; “adalimumab” (Humira® (D2E7)), a human anti-TNF monoclonal containing human-derived heavy chain and light chain variable regions and human IgG:κ constant regions was launched in 2002 for the treatment of rheumatoid arthritis; and, “omalizumab” (Xolair®), which binds to IgE and prevents it from binding to mast cells was approved in 2003 for the treatment of adults and adolescents over 12 years of age with moderate to severe persistent asthma who have a positive skin test or *in vitro* reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids.

Thus, protein engineering has been applied in an effort to diminish problems related to immunogenicity of administered recombinant immunoglobulin polypeptides and to try to alter antibody effector functions. However, problems remain. For example, the majority of cancer patients treated with rituximab relapse, generally within about 6-12 months, and fatal infusion reactions within 24 hours of rituximab infusion have been reported. These fatal reactions followed an infusion reaction complex that included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation or cardiogenic shock. Acute renal failure requiring dialysis with instances of fatal outcome has also been reported in the setting of tumor lysis syndrome following treatment with rituximab, as have severe mucocutaneous reactions, some with fatal outcome. Additionally, high doses of rituximab are required for intravenous injection

because the molecule is large, approximately 150 kDa, and diffusion is limited into the lymphoid tissues where many tumor cells reside.

Trastuzumab administration can result in the development of ventricular dysfunction and congestive heart failure, and the incidence and severity of cardiac  
5 dysfunction has been reported to be particularly high in patients who received trastuzumab in combination with anthracyclines and cyclophosphamide. Trastuzumab administration can also result in severe hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events.

Patients receiving daclizumab immunosuppressive therapy are at increased  
10 risk for developing lymphoproliferative disorders and opportunistic infections, and it is not known whether daclizumab use will have a long-term effect on the ability of the immune system to respond to antigens first encountered during daclizumab-induced immunosuppression.

Hepatotoxicity, including severe hepatic veno-occlusive disease (VOD), has  
15 also been reported in association with the use of gemtuzumab as a single agent, as part of a combination chemotherapy regimen, and in patients without a history of liver disease or hematopoietic stem-cell transplant (HSCT). Patients who receive gemtuzumab either before or after HSCT, patients with underlying hepatic disease or abnormal liver function, and patients receiving gemtuzumab in combinations with other chemotherapy may be at  
20 increased risk for developing severe VOD. Death from liver failure and from VOD has been reported in patients who received gemtuzumab, and it has been cautioned that even careful monitoring may not identify all patients at risk or prevent the complications of hepatotoxicity.

Hepatotoxicity was also reported in patients receiving alemtuzumab. Serious  
25 and, in some rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving alemtuzumab therapy. Alemtuzumab can also result in serious infusion reactions as well as opportunistic infections.

In patients treated with adalimumab, serious infections and sepsis, including  
30 fatalities, have been reported, as has the exacerbation of clinical symptoms and/or

radiographic evidence of demyelinating disease, and patients treated with adalimumab in clinical trials had a higher incidence of lymphoma than the expected rate in the general population.

Serious adverse reactions in clinical studies with omalizumab have included malignancies and anaphylaxis, in which the observed incidence of malignancy among omalizumab-treated patients (0.5%) was numerically higher than among patients in control groups (0.2%).

Smaller immunoglobulin molecules have been constructed in an effort to overcome various problems associated with whole immunoglobulin therapy. Single chain immunoglobulin variable region fragment polypeptides (scFvs) are made of an immunoglobulin heavy chain variable domain joined via a short linker peptide to an immunoglobulin light chain variable domain. Huston *et al.*, 1988 *Proc. Natl. Acad. Sci. USA*, **85**:5879-83. It has been suggested that the smaller size of scFv molecules may lead to more rapid clearance from plasma and more effective penetration into tissues than whole immunoglobulins. See, *e.g.*, Jain, 1990 *Cancer Res.* **50**:814s-819s. An anti-tumor scFv was reported to show more rapid tumor penetration and more even distribution through the tumor mass than the corresponding chimeric antibody. Yokota *et al.*, *Cancer Res.* **52**:3402-08 (1992).

Despite advantages that scFv molecules may have with regard to serotherapy, drawbacks to this therapeutic approach also exist. For example, rapid clearance of scFv may prevent delivery of a minimum effective dose to the target tissue. Additionally, manufacturing adequate amounts of scFv for administration to patients has been challenging due to difficulties in expression and isolation of scFv that adversely affect yields. During expression, scFv molecules lack stability and often aggregate due to pairing of variable regions from different molecules. Furthermore, production levels of scFv molecules in mammalian expression systems are reportedly low, which may limit the potential for efficient manufacturing of scFv molecules for therapy. Davis *et al.*, 1990 *J. Biol. Chem.* **265**:10410-18; Traunecker *et al.*, 1991 *EMBO J.* **10**:3655-59. Strategies for means to improve production have been explored, and reportedly include the addition of glycosylation sites to variable regions. See, *e.g.*, U.S. Patent No. 5,888,773; Jost *et al.*, 1994 *J. Biol.*

*Chem.* **269**:26267-73. Another disadvantage to the use of scFvs for therapy is the lack of effector function. An scFv that lacks the cytolytic functions, ADCC, and complement dependent-cytotoxicity may be less effective or ineffective for treating disease. Even though development of scFv technology began over 12 years ago, there are currently no scFv products approved for therapy.

Alternatively, it has been proposed that fusion of an scFv to another molecule, such as a toxin, could take advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. Chaudary *et al.*, 1989 *Nature* **339**:394; Batra *et al.*, 1991 *Mol. Cell. Biol.* **11**:2200. Conjugation or fusion of toxins to scFvs has thus been offered as an alternative strategy to provide potent, antigen-specific molecules, but dosing with such conjugates or chimeras can be limited by excessive and/or non-specific toxicity due to the toxin moiety of such preparations. Toxic effects may include supraphysiological elevation of liver enzymes and vascular leak syndrome, and other undesired effects. In addition, immunotoxins are themselves highly immunogenic upon administration to a host, and host antibodies generated against the immunotoxin limit potential usefulness for repeated therapeutic treatments of an individual.

Fusion proteins in which immunoglobulin constant region polypeptide sequences are present and nonimmunoglobulin sequences are substituted for the antibody variable regions have also been investigated. For example, CD4, the T cell surface protein recognized by HIV, was recombinantly fused to an immunoglobulin Fc effector domain, and an IL-2-IgG1 fusion protein reportedly effected complement-mediated lysis of IL-2 receptor-bearing cells. See Sensel *et al.*, *Chem. Immunol.* **65**:129-158 (1997).

An extensive introduction as well as detailed information about all aspects of recombinant antibody technology can be found in the textbook "Recombinant Antibodies" (John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab Protocols can be found in R. Kontermann and S. Dübel (eds.), "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000). Diseases and disorders thought to be amenable to some type of immunoglobulin therapy include cancer and immune system disorders. Cancer includes a broad range of diseases, affecting approximately one in four individuals worldwide. Rapid and unregulated

proliferation of malignant cells is a hallmark of many types of cancer, including hematological malignancies. Although patients with a hematologic malignant condition have benefited from advances in cancer therapy in the past two decades, Multani *et al.*, 1998 *J. Clin. Oncology* 16:3691-3710, and remission times have increased, most patients still  
5 relapse and succumb to their disease. Barriers to cure with cytotoxic drugs include, for example, tumor cell resistance and the high toxicity of chemotherapy, which prevents optimal dosing in many patients.

Nevertheless, patients have been treated with immunotherapeutics that target malignant cells, *i.e.*, to antigens expressed on tumor cells.. With regard to the selection of  
10 tumor cell surface antigens suitable for use as immunotherapy targets, it is preferable that such a target antigen is not expressed by normal tissues, particularly where the preservation of such tissue is important to host survival. In the case of hematologic malignancy, malignant cells express many antigens that are not expressed on the surfaces of stem cells or other essential cells. Treatment of a hematologic malignant condition using a therapeutic  
15 regimen that depletes both normal and malignant cells of hematological origin has been acceptable where regeneration of normal cells from progenitors can occur after therapy has ended. Additionally, the target antigen is desirably expressed on all or virtually all clonogenic populations of tumor cells, and it is best that expression persists despite selective pressure from immunoglobulin therapy. Strategies that employ selection of a cell surface  
20 idiotype (*e.g.*, a particular idiotope) as a target for therapy of B cell malignancy have been limited by the outgrowth of tumor cell variants with altered surface idiotype expression, even where the antigen exhibits a high degree of tumor selectivity. Meeker *et al.*, 1985 *N. Engl. J. Med.* 312:1658-65. The selected antigen should also traffic properly after an immunoglobulin binds to it. Shedding or internalization of a cell surface target antigen after  
25 an immunoglobulin binds to the antigen may allow tumor cells to escape destruction, thus limiting the effectiveness of serotherapy. Finally, binding of an immunoglobulin to cell surface target antigens that transmit or transduce cellular activation signals may result in improved functional responses to immunotherapy in tumor cells, and can lead to growth arrest and/or apoptosis. While all of these properties are important, the triggering of



apoptosis after an immunoglobulin binds to the target antigen may also be a critical factor in achieving successful serotherapy.

Antigens that have been tested as targets for serotherapy of B and T cell malignancies include Ig idiotype (Brown *et al.*, 1989 *Blood* 73:651-61), CD19 (Hekman *et al.*, 1991 *Cancer Immunol. Immunother.* 32:364-72), Vlasveld *et al.*, 1995 *Cancer Immunol. Immunother.* 40:37-47), CD20 (Press *et al.*, 1987 *Blood* 69: 584-91), Maloney *et al.*, 1997 *J. Clin. Oncol.* 15:3266-74), CD21 (Scheinberg *et al.*, 1990 *J. Clin. Oncol.* 8:792-803), CD5 (Dillman *et al.*, 1986 *J. Biol. Respn. Mod.* 5:394-410), and CD52 (CAMPATH) (Pawson *et al.*, 1997 *J. Clin. Oncol.* 15:2667-72). Of these, greater benefit for therapy of B cell lymphomas has been obtained using molecules that target CD20. . Other targets have been limited by biological properties of the antigen. For example, surface idiotype can be altered through somatic mutation, allowing tumor cell escape. CD5, CD21, and CD19 are rapidly internalized after monoclonal antibody binding, allowing tumor cells to escape destruction unless monoclonal antibodies are conjugated with toxin molecules. CD22 is expressed on only a subset of B cell lymphomas, thereby limiting its usefulness, while CD52 is expressed on both T cells and B cells and may therefore generate counterproductive immunosuppression by depletion.

Treatment of patients with low grade or follicular B cell lymphoma using a chimeric CD20 monoclonal antibody has been reported to induce partial or complete responses in patients. McLaughlin *et al.*, 1996 *Blood* 88:90a (abstract, suppl. 1); Maloney *et al.*, 1997 *Blood* 90:2188-95. However, as noted above, tumor relapse commonly occurs within six months to one year. Further improvements in serotherapy are needed to induce more durable responses, for example, in low grade B cell lymphoma, and to allow effective treatment of high grade lymphoma and other B cell diseases.

Another approach has been to target radioisotopes to B cell lymphomas using monoclonal antibodies specific for CD20. While the effectiveness of therapy is reportedly increased, associated toxicity from the long *in vivo* half-life of the radioactive antibody increases also, sometimes requiring that the patient undergo stem cell rescue. Press *et al.*, 1993 *N. Eng. J. Med.* 329:1219-1224; Kaminski *et al.*, 1993 *N. Eng. J. Med.* 329:459-65. Monoclonal antibodies to CD20 have also been cleaved with proteases to yield F(ab')<sub>2</sub> or

Fab fragments prior to attachment of radioisotope. This has been reported to improve penetration of the radioisotope conjugate into the tumor and to shorten the *in vivo* half-life, thus reducing the toxicity to normal tissues. However, these molecules lack effector functions, including complement fixation and/or ADCC..

5 CD20 was the first human B cell lineage-specific surface molecule identified by a monoclonal antibody. It is a non-glycosylated, hydrophobic 35 kDa B cell transmembrane phosphoprotein that has both amino and carboxy ends situated in the cytoplasm. Einfeld *et al.*, 1988 *EMBO J.* 7:711-17. CD20 is expressed by all normal mature B cells, but is not expressed by precursor B cells. Natural ligands for CD20 have not been  
10 identified, and the function of CD20 in B cell biology is still incompletely understood.

Anti-CD20 monoclonal antibodies affect the viability and growth and growth of B cells. Clark *et al.*, 1986 *Proc. Natl. Acad. Sci. USA* 83:4494-98. Extensive cross-linking of CD20 can induce apoptosis in B lymphoma cell lines, Shan *et al.*, 1998 *Blood* 91:1644-52, and cross-linking of CD20 on the cell surface has been reported to  
15 increase the magnitude and enhance the kinetics of signal transduction, for example, as detected by measuring tyrosine phosphorylation of cellular substrates. Deans *et al.*, 1993 *J. Immunol.* 146:846-53. Therefore, in addition to cellular depletion by complement and ADCC mechanisms, Fc-receptor binding by CD20 monoclonal antibodies *in vivo* may promote apoptosis of malignant B cells by CD20 cross-linking, consistent with the theory  
20 that effectiveness of CD20 therapy of human lymphoma in a SCID mouse model may be dependent upon Fc-receptor binding by the CD20 monoclonal antibody. Funakoshi *et al.*, 1996 *J. Immunotherapy* 19:93-101. The presence of multiple membrane spanning domains in the CD20 polypeptide (Einfeld *et al.*, 1988 *EMBO J.* 7:711-17; Stamenkovic *et al.*, 1988 *J. Exp. Med.* 167:1975-80; Tedder *et al.*, 1988 *J. Immunol.* 141:4388-4394), prevent CD20  
25 internalization after antibody binding, and this was recognized as an important feature for therapy of B cell malignancies when a murine CD20 monoclonal antibody, 1F5, was injected into patients with B cell lymphoma, resulting in significant depletion of malignant cells and partial clinical responses. Press *et al.*, 1987 *Blood* 69:584-91.

Because normal mature B cells also express CD20, normal B cells are  
30 depleted by anti-CD20 antibody therapy. Reff, M.E. *et al.*, 1994 *Blood* 83:435-445. After

treatment is completed, however, normal B cells can be regenerated from CD20 negative B cell precursors; therefore, patients treated with anti-CD20 therapy do not experience significant immunosuppression. Depletion of normal B cells may also be beneficial in diseases that involve inappropriate production of autoantibodies or other diseases where B cells may play a role. A chimeric monoclonal antibody specific for CD20, consisting of heavy and light chain variable regions of mouse origin fused to human IgG1 heavy chain and human kappa light chain constant regions, reportedly retained binding to CD20 and the ability to mediate ADCC and to fix complement. Liu *et al.*, 1987 *J. Immunol.* **139**:3521-26. The mechanism of anti-tumor activity of rituximab, discussed above, is thought to be a combination of several activities, including ADCC, complement fixation, and triggering of signals that promote apoptosis in malignant B cells, although the large size of rituximab prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B cells, thereby limiting these anti-tumor activities. Autoimmune diseases include autoimmune thyroid diseases, which include Graves' disease and Hashimoto's thyroiditis. In the United States alone, there are about 20 million people who have some form of autoimmune thyroid disease. Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis). Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor. Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens. Current therapy for Graves' disease includes surgery, radioactive iodine, or antithyroid drug therapy. Radioactive iodine is widely used, since antithyroid medications have significant side effects and disease recurrence is high. Surgery is reserved for patients with large goiters or where there is a need for very rapid normalization of thyroid function. There are no therapies that target the production of autoantibodies responsible for stimulating the TSH receptor. Current therapy for Hashimoto's thyroiditis is levothyroxine sodium, and therapy is usually lifelong because of the low likelihood of remission. Suppressing therapy has been shown to shrink goiters in Hashimoto's thyroiditis, but no therapies that reduce autoantibody production to target the disease mechanism are known.

Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints, leading to swelling, pain, and loss of function. RA affects an estimated 2.5 million people in the United States. RA is caused by a combination of events including an initial infection or injury, an abnormal immune response, and genetic factors. While  
5 autoreactive T cells and B cells are present in RA, the detection of high levels of antibodies that collect in the joints, called rheumatoid factor, is used in the diagnosis of RA. Current therapy for RA includes many medications for managing pain and slowing the progression of the disease. No therapy has been found that can cure the disease. Medications include nonsteroidal antiinflammatory drugs (NSAIDs), and disease modifying antirheumatic drugs  
10 (DMARDS). NSAIDs are useful in benign disease, but fail to prevent the progression to joint destruction and debility in severe RA. Both NSAIDs and DMARDS are associated with significant side effects. Only one new DMARD, Leflunomide, has been approved in over 10 years. Leflunomide blocks production of autoantibodies, reduces inflammation, and slows progression of RA. However, this drug also causes severe side effects including  
15 nausea, diarrhea, hair loss, rash, and liver injury.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. SLE is estimated to affect over 500,000 people in the United States. In patients with SLE, a faulty interaction between T cells and B cells results in the production of autoantibodies that  
20 attack the cell nucleus. These include anti-double stranded DNA and anti-Sm antibodies. Autoantibodies that bind phospholipids are also found in about half of SLE patients, and are responsible for blood vessel damage and low blood counts. Immune complexes accumulate the kidneys, blood vessels, and joints of SLE patients, where they cause inflammation and tissue damage. No treatment for SLE has been found to cure the disease. NSAIDs and  
25 DMARDS are used for therapy depending upon the severity of the disease. Plasmapheresis with plasma exchange to remove autoantibodies can cause temporary improvement in SLE patients. There is general agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B cell lineage, allowing the immune system to reset as new B cells are generated from precursors, would offer hope for long lasting benefit in SLE patients.

Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands. Sjogren's syndrome is one of the most prevalent autoimmune disorders, striking up to an estimated 4 million people in the United States. About half of people stricken with Sjogren's syndrome also have a connective tissue disease, such as RA, while the other half have primary Sjogren's syndrome with no other concurrent autoimmune disease. Autoantibodies, including anti-nuclear antibodies, rheumatoid factor, anti-fodrin, and anti-muscarinic receptor are often present in patients with Sjogren's syndrome. Conventional therapy includes corticosteroids, and additional more effective therapies would be of benefit.

Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction. Some cases of ITP are caused by drugs, and others are associated with infection, pregnancy, or autoimmune disease such as SLE. About half of all cases are classified as "idiopathic", meaning the cause is unknown. The treatment of ITP is determined by the severity of the symptoms. In some cases, no therapy is needed although in most cases immunosuppressive drugs, including corticosteroids or intravenous infusions of immune globulin to deplete T cells, are provided. Another treatment that usually results in an increased number of platelets is removal of the spleen, the organ that destroys antibody-coated platelets. More potent immunosuppressive drugs, including cyclosporine, cyclophosphamide, or azathioprine are used for patients with severe cases. Removal of autoantibodies by passage of patients' plasma over a Protein A column is used as a second line treatment in patients with severe disease. Additional more effective therapies are desired.

Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflammation of the central nervous system and destruction of myelin, which insulates nerve cell fibers in the brain, spinal cord, and body. Although the cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebral spinal fluid of patients with MS, and some theories predict that the B cell response leading to antibody production is important for mediating the disease. No B cell depletion therapies have been studies in patients with MS, and there is no cure for MS. Current therapy is corticosteroids,

which can reduce the duration and severity of attacks, but do not affect the course of MS over time. New biotechnology interferon (IFN) therapies for MS have recently been approved but additional more effective therapies are desired.

Myasthenia Gravis (MG) is a chronic autoimmune neuromuscular disorder that is characterized by weakness of the voluntary muscle groups. MG affects about 40,000 people in the United States. MG is caused by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions. The autoantibodies reduce or block acetylcholine receptors, preventing the transmission of signals from nerves to muscles. There is no known cure for MG. Common treatments include immunosuppression with corticosteroids, cyclosporine, cyclophosphamide, or azathioprine. Surgical removal of the thymus is often used to blunt the autoimmune response. Plasmapheresis, used to reduce autoantibody levels in the blood, is effective in MG, but is short-lived because the production of autoantibodies continues. Plasmapheresis is usually reserved for severe muscle weakness prior to surgery. New and effective therapies would be of benefit.

Psoriasis affects approximately five million people, and is characterized by autoimmune inflammation in the skin. Psoriasis is also associated with arthritis in 30% (psoriatic arthritis). Many treatments, including steroids, UV light, retinoids, vitamin D derivatives, cyclosporine, methotrexate have been used but it is also plain that psoriasis would benefit from new and effective therapies. Scleroderma is a chronic autoimmune disease of the connective tissue that is also known as systemic sclerosis. Scleroderma is characterized by an overproduction of collagen, resulting in a thickening of the skin, and approximately 300,000 people in the United States have scleroderma, which would also benefit from new and effective therapies.

There is a clear need for improved compositions and methods to treat malignancies, including B cell malignancies and disorders including autoimmune diseases, disorders, and conditions, as well as other diseases, disorders, and conditions. The compositions and methods of the present invention described and claimed herein provide such improved compositions and methods as well as other advantages.

## SUMMARY OF THE INVENTION

It is an aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE. The binding domain-immunoglobulin fusion protein further comprises a region that comprises, consists essentially of, or consists of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE). Such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation. Such binding domain polypeptides are also capable of binding or specifically binding to a target, for example, a target antigen.

In certain embodiments, for example, the binding domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In certain further embodiments the binding domain-immunoglobulin fusion protein comprises a native or engineered immunoglobulin heavy chain variable region polypeptide, wherein the heavy chain variable region polypeptide is an engineered human immunoglobulin heavy chain variable region polypeptide (or an engineered immunoglobulin heavy chain variable region polypeptide from a non-human species) comprising a mutation, substitution, or deletion of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108, 110, and/or 112. Mutations, substitutions, or deletions of an amino acid(s) at a location corresponding to any one or more of amino acid positions 9, 10, 11, 12, 108,

110, and/or 112 in a heavy chain variable region may be included within a construct such as the construct corresponding to, for example, SEQ ID NO:\_\_\_\_. In certain other further embodiments the fusion protein comprises a polypeptide having a sequence selected from SEQ ID NOS:\_\_\_ or SEQ ID NO:\_\_\_\_. In certain embodiments the immunoglobulin variable region polypeptide is derived from, for example, a human immunoglobulin, and in certain other embodiments the immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence. In certain embodiments the immunoglobulin variable region polypeptide, whether or not humanized, is derived from a murine immunoglobulin, or is derived from an immunoglobulin from another species, including, for example a rat, a pig, a monkey, or a camelid.

According to certain embodiments of the present invention, the binding domain polypeptide comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide; and (c) at least one linker polypeptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b). In certain further embodiments the native or engineered immunoglobulin light chain variable region and heavy chain variable region polypeptides are derived or constructed from human immunoglobulins, and in certain other further embodiments the linker polypeptide comprises at least one polypeptide including or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:\_\_\_\_]. In other embodiments the linker polypeptide comprises at least two or three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:\_\_\_\_]. In other embodiments the linker comprises a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide is derived from an IgG or IgA human immunoglobulin heavy chain. In another embodiment at least one of a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide and a



native or engineered immunoglobulin heavy chain CH4 constant region polypeptide is derived from an IgE human immunoglobulin heavy chain.

An immunoglobulin hinge region polypeptide may comprise, consist essentially or, or consist of, for example, any of (1) any hinge or hinge-acting peptide or polypeptide that occurs naturally for example, a human immunoglobulin hinge region polypeptide including, for example, a wild-type human IgG hinge or a portion thereof, a wild-type human IgA hinge or a portion thereof, a wild-type human IgD hinge or a portion thereof, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2, or a portion thereof, a wild-type camelid hinge region or a portion thereof (including a IgG1 llama hinge region or portion thereof, a IgG2 llama hinge region or portion thereof, and a IgG3 llama hinge region or portion thereof), a nurse shark hinge region or portion thereof, and/or a spotted ratfish hinge region or a portion thereof; (2) a mutated or otherwise altered or engineered hinge region polypeptide that contains no cysteine residues and that is derived or constructed from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues; (3) a mutated or otherwise altered or engineered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region polypeptide having one or more cysteine residues; (4) a hinge region polypeptide that has been mutated or otherwise altered or engineered to contain or add one or more glycosylation sites, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site; (5) a mutated or otherwise altered or engineered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid substitution or deletion, for example, a mutated or otherwise altered or engineered IgG1 or IgG4 hinge region containing for example zero, one, or two cysteine residues, a mutated or otherwise altered or engineered IgG2 hinge region containing for example zero, one, two or three cysteine residues, a mutated or otherwise altered or engineered IgG3 hinge region containing for example zero, one, two, three, or from four to ten cysteine residues, or a mutated or otherwise altered or engineered human IgA1 or IgA2 hinge region polypeptide that contains zero or only one or two cysteine residues (*e.g.*, an "SCC" hinge), a mutated or otherwise altered or engineered IgD hinge region containing no cysteine residues, or a mutated or otherwise altered or engineered

human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide that contains zero or only one, two, three or four cysteine residues; or (6) any other connecting region molecule described or referenced herein or otherwise known or later discovered as useful for connecting adjoining immunoglobulin domains such as, for example, a CH1 domain and a CH2 domain. For example, a hinge region polypeptide may be selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, for example, (ii) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived or constructed from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 or other immunoglobulin hinge region polypeptide contains no more than one cysteine residue, and (iv) a mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated or otherwise altered or engineered human IgG1 or other immunoglobulin hinge region polypeptide contains no cysteine residues. In certain embodiments, for example, the immunoglobulin hinge region polypeptide is a mutated or otherwise altered or engineered hinge region polypeptide and exhibits a reduced ability to dimerize, relative to a wild-type human immunoglobulin G or other wild type hinge region or hinge-acting polypeptide.

The immunoglobulin heavy chain constant region polypeptides may be, for example, native or engineered CH2 and CH3 domains of an isotype that is human IgG or human IgA. The immunoglobulin heavy chain constant region polypeptides may also be, for example, native or engineered immunoglobulin heavy chain constant region CH3 and CH4 polypeptides of an isotype that is human IgE.

In certain other embodiments the target or target antigen may be, for example, CD19 (B-lymphocyte antigen CD19, also referred to as B-lymphocyte surface

antigen B4, or Leu-12), CD20 (B-lymphocyte antigen 20, also referred to as B-lymphocyte surface antigen B1, Leu-16, or Bp35), CD22 (B-cell receptor CD22, also referred to as Leu-14, B-lymphocyte cell adhesion molecule, or BL-CAM), CD37 (leukocyte antigen CD37), CD40 (B-cell surface antigen CD40, also referred to as Tumor Necrosis Factor receptor superfamily member 5, CD40L receptor, or Bp50), CD80 (T lymphocyte activation antigen CD80, also referred to as Activation B7-1 antigen, B7, B7-1, or BB1), CD86 (T lymphocyte activation antigen CD86, also referred to as Activation B7-2 antigen, B70, FUN-1, or BU63), CD137 (also referred to as Tumor Necrosis Factor receptor superfamily member 9), CD152 (also referred to as cytotoxic T-lymphocyte protein 4 or CTLA-4), CD45 (Leukocyte common antigen, also referred to as L-CA, T200, and EC 3.1.3.48), CD45RA (an isoform of CD45, and an antigen expressed on naïve or immature lymphocytes), CD45RB (an isoform of CD45), CD45RO (an isoform of CD45, and a common leukocyte antigen expressed on memory B and T cells), L6 (Tumor-associated antigen L6, also referred to as Transmembrane 4 superfamily member 1, Membrane component surface marker 1, or M3S1), CD2 (T-cell surface antigen CD2, also referred to as T-cell surface antigen T11/Leu-5, LFA-2, LFA-3 receptor, Erythrocyte receptor, or Rosette receptor), CD28 (T-cell-specific homodimer surface protein CD28, also referred to as Tp44), CD30 (lymphocyte activation antigen CD30, also referred to as Tumor Necrosis Factor receptor superfamily member 8, CD30L receptor, or Ki-1), CD50 (also referred to as Intercellular adhesion molecule-3 (ICAM3), or ICAM-R), CD54 (also referred to as Intercellular adhesion molecule-1 (ICAM1), or Major group rhinovirus receptor), B7-H1 (ligand for an immunoinhibitory receptor expressed by activated T cells, B cells, and myeloid cells, also referred to as PD-L1; see Dong, *et al.*, "B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion," 1999 *Nat. Med.* 5:1365-1369), CD134 (also referred to as Tumor Necrosis Factor receptor superfamily member 4, OX40, OX40L receptor, ACT35 antigen, or TAX-transcriptionally activated glycoprotein 1 receptor), 41BB (4-1BB ligand receptor, T-cell antigen 4-1BB, or T-cell antigen ILA), CD153 (also referred to as Tumor Necrosis Factor ligand superfamily member 8, CD30 ligand, or CD30-L), CD154 (also referred to as Tumor Necrosis Factor ligand superfamily member 5, CD40 ligand, CD40-L, TNF-related activation protein, TRAP, or T cell antigen

Gp39), ICOS (Inducible Costimulator), CD3 (one or more of the delta, epsilon, gamma, eta and/or zeta chains, alone or in combination), CD4 (T-cell surface glycoprotein CD4, also referred to as T-cell surface antigen T4/Leu-3), CD25 (also referred to as Interleukin-2 receptor alpha chain, IL-2 receptor alpha subunit, p55, or Tac antigen), CD8 $\alpha$  (T-cell surface glycoprotein CD8 alpha chain, also referred to as T-lymphocyte differentiation antigen, T8/Leu-2, and Lyt-2), CD11b (also referred to as Integrin alpha-M, Cell surface glycoprotein MAC-1 alpha subunit, CR-3 alpha chain, Leukocyte adhesion receptor Mo1, or Neutrophil adherence receptor), CD14 (Monocyte differentiation antigen CD14, also referred to as Myeloid cell-specific leucine-rich glycoprotein or LPS receptor), CD56 (also referred to as Neural cell adhesion molecule 1), or CD69 (also referred to as Early T-cell activation antigen p60, Gp32/28, Leu-23, MLR-3, Activation inducer molecule, or AIM). The above list of construct targets and/or target antigens is exemplary only and is not exhaustive.

In another aspect, the invention includes a binding construct (or a polynucleotide encoding such a construct) that comprises a CD154 extracellular domain, or desired functional portion thereof. In one embodiment of this aspect of the invention, for example, the binding construct comprises a CD154 extracellular domain fused or otherwise connected to a second binding domain. The second binding domain, for example, may comprise, consist essentially of, or consist of at least one immunoglobulin variable region polypeptide. The at least one immunoglobulin variable region polypeptide may be a native or engineered scFv. The native or engineered scFv may be a native or engineered scFv disclosed or described herein. The second binding domain, including a native or engineered scFv, may be one that binds, for example, to any of the targets, including target antigens, disclosed or described herein, including but not limited to, for example, any of B7-H1, ICOS, L6, CD2, CD3, CD8, CD4, CD11b, CD14, CD19, CD20, CD22, CD25, CD28, CD30, CD37, CD40, CD45, CD50, CD54, CD56, CD69, CD80, CD86, CD134, CD137, CD152, CD153, or CD154.

In another embodiment the binding domain polypeptide comprises a CTLA-4 extracellular domain, or desired functional portion thereof, and in further embodiments at least one of the immunoglobulin heavy chain constant region polypeptides selected from a

CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide, either native or engineered.

In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a  
5 CH3 constant region polypeptide is a human IgA constant region polypeptide, either native or engineered.

In another further embodiment at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH3 constant region polypeptide and a  
10 CH4 constant region polypeptide is a human IgE constant region polypeptide, either native or engineered.

Turning to another embodiment, the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin  
15 heavy chain CH2 (or IgE Ch3) constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide that is fused or otherwise connected to the CH2 (or IgE CH3) constant region polypeptide, wherein (1) the binding domain polypeptide comprises a CTLA-4 extracellular domain, or a portion thereof, that is  
20 capable of binding or specifically binding to at least one CTLA-4 ligand selected from the group consisting of CD80 and CD86, (2) the immunoglobulin hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide that is selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG1 hinge region  
25 polypeptide, and a native or engineered human IgE CH2 region polypeptide (3) a immunoglobulin heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH2 constant region polypeptide, a native or engineered human IgG1 heavy chain CH2 constant region polypeptide, and a native or  
30 engineered human IgE heavy chain CH3 constant region polypeptide (4) a immunoglobulin

heavy chain constant region polypeptide that comprises, consists essentially of, or consists of, a polypeptide that is selected from the group consisting of a native or engineered human IgA heavy chain CH3 constant region polypeptide, a native or engineered human IgG1 heavy chain CH3 constant region polypeptide, and a native or engineered human IgE heavy chain CH4 constant region polypeptide, and (5) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation.

In another embodiment the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a native or engineered human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first native or engineered constant region polypeptide, wherein said native or engineered second constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgE CH4 constant region polypeptide and wherein (1) the binding domain-immunoglobulin fusion protein is capable of inducing at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In a further embodiment the antigen is a tumor antigen.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an

immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide

5 selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said first native or

10 engineered constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is

15 fused or otherwise connected to the first constant region polypeptide, wherein said second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, and a native or engineered human IgE CH4 constant region polypeptide; and (d) a native or

20 engineered plasma membrane anchor domain polypeptide. In one example of this embodiment, the plasma membrane anchor domain polypeptide links to a membrane via a native or engineered glycosyl-phosphatidylinositol-linkage. In a further embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide. In another further

25 embodiment the membrane anchor domain polypeptide comprises, consists essentially of, or consists of, a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide. In a still further embodiment the cytoplasmic tail polypeptide comprises, consists essentially of, or consists of, a native or engineered apoptosis signaling polypeptide sequence, which in a still further embodiment is derived or

30 constructed from a native or engineered receptor death domain polypeptide, a death domain,

or a functional portion of either. In a further embodiment the native or engineered death domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered polypeptide selected from an ITIM domain (immunoreceptor Tyr-based inhibition motif), an ITAM domain (immunoreceptor Tyr-based activation motif), TRAF, RIP, CRADD, FADD (Fas-associated death domain), TRADD (Tumor Necrosis Factor receptor type 1 associated DEATH domain protein), RAIDD (also referred to as RAID), CD95 (Tumor Necrosis Factor receptor superfamily member 6, also referred to as FASL receptor, Apoptosis-mediating surface antigen FAS, FAS and Apo-1 antigen), TNFR1, and/or DR5 (death receptor-5). In another embodiment the native or engineered apoptosis signaling polypeptide sequence comprises, consists essentially of, or consists of, for example, a polypeptide sequence derived from a native or engineered caspase polypeptide that is caspase-3 or caspase-8 or caspase-10, including caspase 8/FLICE/MACH/Mch5 and caspase 10/Flice2/Mch4. In another embodiment the plasma membrane anchor domain polypeptide comprises, consists essentially of, or consists of, for example, a native or engineered glycosyl-phosphatidylinositol-linkage polypeptide sequence. In another embodiment the antigen that is present on an immune effector cell is, for example, CD2, CD16, CD28, CD30, CD32, CD40, CD50, CD54, CD64, CD80, CD86, B7-H1, CD134, CD137, CD152, CD153, CD154, ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD56, or CD69. In another embodiment the human IgG is a native or engineered human IgG1. These binding domain-immunoglobulin fusion proteins may be capable of inducing, for example, at least one immunological activity selected from antibody dependent cell-mediated cytotoxicity and/or complement fixation and/or CDC, and are capable of binding or specifically binding to a target, including, for example, a target antigen. Immune effector cells include, for example, granulocytes, mast cells, monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils, NK cells, T cells (including Th1 cells, Th2 cells, Tc cells, memory T cells, null cells, and large granular lymphocytes, *etc.*), and B cells. This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such vectors for *in vivo* and *ex vivo* gene therapy. The above lists of construct components and targets are not exhaustive and



may include any desired target or component that may function as, or be useful for the purposes, described herein.

In another embodiment, the invention provides a protein having a first protein motif that comprises, consists essentially of, or consists of, (1) a native or engineered immunoglobulin hinge region or hinge-acting region (*e.g.*, IgE CH2) polypeptide that is fused or otherwise connected to (2) a native or engineered CH2 constant region polypeptide (or native or engineered IgE CH3 constant region polypeptide). Said first protein motif may be fused or otherwise connected to one or more other such first protein motifs to form a second protein motif, the second protein motif being fused or otherwise connected to (3) a native or engineered CH3 constant region (or a native or engineered IgE CH4 constant region) to form a third protein motif. Said first, second or third protein motifs may be fused or otherwise connected to one or more of the herein-described native or engineered plasma membrane anchor domain polypeptides, including, for example, a native or engineered transmembrane domain polypeptide, and a native or engineered transmembrane domain polypeptide and a native or engineered cytoplasmic tail polypeptide, such as for example, a native or engineered apoptosis signaling polypeptide sequence, which may be derived or constructed from a native or engineered receptor death domain polypeptide, a death domain, or a functional portion of either. Thus, a protein or polynucleotide within this aspect of the invention may be, for example, a Hinge-CH2-CH3-TransmembraneDomain-DeathDomain construct. It may also be, for example, a (Hinge-CH2)<sub>X</sub>-CH3- TransmembraneDomain-DeathDomain construct, where X is from 2 to about 5, or such other number as may be needed to achieve a desired length or Fc receptor binding and/or complement fixation function(s). This embodiment of the invention also includes polynucleotides encoding such proteins, vectors including such polynucleotides, and host cells containing such polynucleotides and vectors. This embodiment of the invention further includes the use of such proteins for therapy, and, for example, the use of such polynucleotides and/or vectors for *in vivo* and *ex vivo* gene therapy. The invention provides, in another embodiment, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is

capable of binding or specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, a polypeptide selected from the group consisting of a native or engineered human IgA hinge region polypeptide, a  
5 native or engineered human IgG hinge region polypeptide, and a native or engineered human IgE hinge-acting region, *i.e.*, IgE CH2, region polypeptide; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein the first constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or  
10 engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, or a native or engineered human IgE CH3 constant region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially  
15 of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In a further embodiment the human IgG polypeptides are native or engineered human IgG1 polypeptides.

In another embodiment the present invention provides a binding domain-  
20 immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of, (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide may be as described above or herein, and may comprises, consist essentially of, or consist of, for example, a wild-type or engineered human IgA hinge region polypeptide; (b) a native or engineered  
25 immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgA CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise  
30 connected to the native or engineered CH2 constant region polypeptide, wherein the native

or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is (i) a wild-type human IgA CH3 constant region polypeptide or other IgA region, preferably human or humanized, that is capable of associating with J Chain, (ii) a mutated, altered or otherwise engineered human IgA CH3 constant region polypeptide that is, for example, incapable of associating with a J chain, wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target such as, for example, an antigen. In certain further embodiments the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is (i) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO:\_\_\_ or (ii) a polypeptide comprising, consisting essentially of, or consisting of, an amino acid sequence as set forth in SEQ ID NO:\_\_\_ . In other embodiments, the IgA hinge region polypeptide is a native or engineered IgA1 hinge region polypeptide or a native or engineered IgA2 hinge region polypeptide. In still other embodiments, the IgA hinge region polypeptide is different from a wild-type IgA1 or IgA2 hinge region polypeptide by, for example, the alteration, substitution, or deletion of one or more of the cysteine residues within said wild-type hinge region.

In certain other embodiments the present invention provides a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein the native or engineered CH2 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH2 constant region polypeptide that is a native or engineered llama IgG1 CH2 constant region polypeptide, a native or engineered llama IgG2 CH2 constant region polypeptide, or a native or engineered llama IgG3 CH2 constant region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise

connected to the native or engineered CH2 constant region polypeptide, wherein said native or engineered CH3 constant region polypeptide comprises, consists essentially of, or consists of, a native or engineered llama CH3 constant region polypeptide that is selected from the group consisting of a native or engineered llama IgG1 CH3 constant region polypeptide, a native or engineered llama IgG2 CH3 constant region polypeptide and a native or engineered llama IgG3 CH3 constant region polypeptide wherein (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, fixation of complement and CDC, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, for example a target antigen. In a further embodiment the immunoglobulin hinge region polypeptide, the native or engineered llama CH2 constant region polypeptide and the native or engineered llama CH3 constant region polypeptide comprise sequences derived from a native or engineered llama IgG1 polypeptide and the fusion protein does not include a native or engineered llama IgG1 CH1 domain. In certain embodiments the invention provides any of the above described binding domain-immunoglobulin fusion proteins wherein the hinge region polypeptide is mutated, engineered, or otherwise altered to contain a glycosylation site, which in certain further embodiments is an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site or a phosphoglycation site.

In certain embodiments the invention, there are provided any of the above or herein described binding constructs, including binding domain-immunoglobulin fusion proteins, wherein a binding region or binding domain polypeptide comprises two or more binding domain polypeptide sequences wherein each of the binding domain polypeptide sequences is capable of binding or specifically binding to a target(s) such as an antigen(s), which target(s) or antigen(s) may be the same or may be different. A native, for more preferably an engineered, IgD hinge is a desired connecting region between binding domains of a bispecific molecule of the invention, *i.e.*, one with two or more binding domains, preferably two. The wild type human IgD hinge has one cysteine that forms a disulfide bond with the light chain in the native IgD structure. It is desirable to mutate or delete this cysteine in the human IgD hinge for use as a connecting region between binding domains of,

for example, a bispecific molecule. Other amino acid changes or deletions or alterations in an IgD hinge that do not result in undesired hinge inflexibility are within the scope of the invention. native or engineered IgD hinge regions from other species are also within the scope of the invention, as are humanized native or engineered IgD hinges from non-human species. The present invention also provides, in certain embodiments, a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH2 constant region polypeptide (or an IgE CH3 constant region polypeptide) that is fused or otherwise connected to the hinge region polypeptide; and (c) a second native or engineered immunoglobulin heavy chain constant region, such as an IgG or IgA CH3 constant region polypeptide (or an IgE CH4 constant region polypeptide) that is fused or otherwise connected to the first constant region polypeptide, wherein: (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, CDC, and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to a target, such as an antigen.

Turning to another embodiment there is provided a binding domain-immunoglobulin fusion protein, comprising, consisting essentially of, or consisting of (a) a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of binding or specifically binding to at least one target, such as an antigen, that is present on a cancer cell surface and wherein the hinge region polypeptide may be as described above or herein, and may comprise, consist essentially of, or consist of, for example, an alternative hinge region polypeptide sequence; (b) a first native or engineered immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide, wherein said native or engineered constant region polypeptide comprises, consists

essentially of, or consists of, a polypeptide selected from the group consisting of a native or engineered human IgA CH2 constant region polypeptide, a native or engineered human IgG CH2 constant region polypeptide, and a native or engineered human IgE CH3 constant region polypeptide; and (c) a second immunoglobulin heavy chain constant region polypeptide that is fused or otherwise connected to the first constant region polypeptide, wherein the second constant region polypeptide comprises, consists essentially of, or consists of, a polypeptide that is a native or engineered human IgA CH3 constant region polypeptide, a native or engineered human IgG CH3 constant region polypeptide, or a native or engineered human IgE CH4 constant region polypeptide. In certain further embodiments the alternative hinge region polypeptide sequence comprises, consists essentially of, or consists of, a polypeptide sequence of at least ten continuous amino acids that are present in a sequence selected from SEQ ID NOS: \_\_-\_\_.

In certain embodiments the present invention provides polynucleotides or vectors (including cloning vectors and expression vectors) or transformed or transfected cells, including isolated or purified or pure polynucleotides, vectors, and isolated transformed or transfected cells, encoding or containing any one of the above or herein described polypeptide or protein constructs of the invention, for example, including binding domain-immunoglobulin fusion proteins. Thus, in various embodiments the invention provides a recombinant cloning or expression construct comprising any such polynucleotide that is operably linked to a promoter.

In other embodiments there is provided a host cell transformed or transfected with, or otherwise containing, any such recombinant cloning or expression construct. Host cells include the cells of a subject undergoing *ex vivo* cell therapy including, for example, *ex vivo* gene therapy.

In a related embodiment there is provided a method of producing a polypeptide or protein or other construct of the invention, for example, including a binding domain-immunoglobulin fusion protein, comprising the steps of (a) culturing a host cell as described or provided for herein under conditions that permit expression of the construct, for example, a binding domain-immunoglobulin fusion protein; and (b) isolating the construct,

for example, the binding domain-immunoglobulin fusion protein from the host cell or host cell culture.

In another embodiment there is provided a pharmaceutical composition comprising any one of the above or herein described polypeptide or protein or other  
5 constructs of the invention, for example (including, for example, binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier.

In another embodiment the invention provides a pharmaceutical composition comprising, for example, an isolated, purified, or pure polynucleotide encoding any one of the polypeptide or protein constructs of the invention, for example (including, for example,  
10 binding domain-immunoglobulin fusion proteins), in combination with a physiologically acceptable carrier, or for example, in combination with, or in, a gene therapy delivery vehicle or vector.

In another embodiment the invention provides a method of treating a subject having or suspected of having a malignant condition or a B cell disorder, comprising  
15 administering to a patient a therapeutically effective amount of any of the pharmaceutical compositions described or claimed herein.

In certain further embodiments the malignant condition or B cell disorder is a B cell lymphoma or B cell leukemia, or a disease characterized by autoantibody production, and in certain other further embodiments the B cell disorder is, for example, rheumatoid  
20 arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis or an autoimmune disease. In certain other embodiments the malignant condition is, for example, melanoma, myeloma, glioma, astrocytoma, lymphoma, leukemia, carcinoma, or sarcoma, and so on.

It is another aspect of the present invention to provide a binding domain-immunoglobulin fusion protein, comprising, consisting essentially or, or consisting of, (a) a  
25 binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is as described herein, and may be selected from the group consisting of (i) a mutated, engineered or otherwise altered hinge region polypeptide that contains no cysteine residues and that is derived from a wild-  
30 type immunoglobulin hinge region polypeptide having one or more cysteine residues, (ii) a

mutated, engineered or otherwise altered hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type immunoglobulin hinge region polypeptide having two or more cysteine residues, (iii) a wild-type human IgA hinge region polypeptide, (iv) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains no cysteine residues, (v) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains one cysteine residue and (vi) a mutated, engineered or otherwise altered human IgA hinge region polypeptide that contains two cysteine residues; (b) a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide that is fused or otherwise connected to the hinge region polypeptide; and (c) a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide that is fused or otherwise connected to the CH2 constant region polypeptide, wherein: (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and (2) the binding domain polypeptide is capable of binding or specifically binding to an antigen. In one embodiment the immunoglobulin hinge region polypeptide is a mutated hinge region polypeptide, for example, and the resulting construct exhibits a reduced ability to dimerize, relative to a construct containing a wild-type human immunoglobulin G hinge region polypeptide. In another embodiment the binding domain polypeptide comprises, consists essentially of, or consists of, at least one native or engineered immunoglobulin variable region polypeptide that is a native or engineered immunoglobulin light chain variable region polypeptide and/or a native or engineered immunoglobulin heavy chain variable region polypeptide. In a further embodiment the native or engineered immunoglobulin variable region polypeptide is derived from a human immunoglobulin and, for example, may be humanized.

In another embodiment, the invention provides a binding domain-immunoglobulin fusion protein includes a binding domain polypeptide that comprises, consists essentially of, or consists of, (a) at least one native or engineered immunoglobulin light chain variable region polypeptide; (b) at least one native or engineered immunoglobulin heavy chain variable region polypeptide; and (c) at least one linker peptide that is fused or otherwise connected to the polypeptide of (a) and to the polypeptide of (b).



In a further embodiment the native or engineered immunoglobulin light chain variable region and the native or engineered heavy chain variable region polypeptides are derived from human immunoglobulins and may, for example, be humanized.

In another embodiment at least one of the native or engineered immunoglobulin heavy chain CH2 (or IgE CH3) constant region polypeptide and the native or engineered immunoglobulin heavy chain CH3 (or IgE CH4) constant region polypeptide is derived or constructed from a human immunoglobulin heavy chain. In another embodiment the native or engineered immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of, or are derived or otherwise prepared or constructed from, an isotype selected from human IgG and human IgA. In another embodiment the target, for example, the target antigen is selected from the group consisting of CD16, CD19, CD20, CD37, CD40, CD45RO, CD80, CD86, CD137, CD152, and L6. In certain further embodiments of the above described fusion protein construct, the binding domain comprises, consists essentially of, or consists of, an scFv and the scFv contains a linker polypeptide that comprises, consists essentially of, or consists of, at least one polypeptide comprising or having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:\_\_\_], and in certain other embodiments the linker polypeptide comprises, consists essentially of, or consists of, at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO:\_\_\_]. In certain embodiments the immunoglobulin hinge region polypeptide comprises, consists essentially of, or consists of, a native or engineered human IgG, IgA, IgD hinge region polypeptide, or a native or engineered IgE CH2 region polypeptide. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain. In certain embodiments the binding domain polypeptide comprises, consists essentially of, or consists of, a native or engineered CD154 extracellular domain and at least one a native or engineered immunoglobulin variable region polypeptide.

In other embodiments the invention provides an isolated polynucleotide encoding any of the constructs of the invention, for example, protein or polypeptide constructs of the invention including binding domain-immunoglobulin fusion proteins, and in related embodiments the invention provides a recombinant expression construct

comprising such a polynucleotide, and in certain further embodiments the invention provides a host cell transformed or transfected with, or otherwise containing, such a recombinant expression construct. In another embodiment the invention provides a method of producing a construct of the invention, for example, a protein or polypeptide construct of the invention such as a binding domain-immunoglobulin fusion protein, comprising the steps of (a) culturing a host cell that has been transformed or transfected with, or otherwise made to contain, a polynucleotide construct of the invention under conditions that permit expression of the construct, for example, a construct encoding a binding domain-immunoglobulin fusion protein; and (b) isolating the construct, for example, the binding domain-immunoglobulin fusion protein, from the host cell culture.

The inventions described and claimed herein include novel molecules useful, for example, as therapeutics and other purposes including diagnostic and research purposes. Such molecules have, for example, antigen-binding or other binding function(s) and one or more effector functions. DNA constructs of the invention are useful in, for example, gene therapies, including *in vivo* and *ex vivo* gene therapies.

In one aspect, various constructs of the molecules of the invention include molecules comprising a “binding region”, a “tail” region, and a “connecting” region that joins a binding region and a tail region.

Binding regions within the molecules of the invention may comprise, for example, binding domains for desired targets, including antigen-binding targets. Binding domains for antigen binding targets may comprise, for example, single chain Fvs and scFv domains. In certain embodiments, molecules of the invention may comprise a binding region having at least one immunoglobulin variable region polypeptide, which may be a light chain or a heavy chain variable region polypeptide. In certain embodiments, molecules of the invention may comprise at least one such light chain V-region and one such heavy chain V-region and at least one linker peptide that connects the V-regions. ScFvs useful in the invention also include those with chimeric binding or other domains or sequences. Other ScFvs useful in the invention also include those with humanized binding or other domains or sequences. In such embodiments, all or a portion of an immunoglobulin binding or other sequence that is derived from a non-human source may be “humanized” according to

recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign.

Example of scFvs useful in the invention, whether included as murine or  
5 other scFvs (including human scFvs), chimeric scFvs, or humanized scFvs, in whole or in part, include anti-human CD20 scFvs (for example, "2H7" scFvs), anti-human CD37 scFvs (for example, "G28-1" scFvs), anti-human CD40 scFvs (for example, "G28-5" scFvs and "40.2.220" scFvs), anti-carcinoma-associated antigen scFvs (for example, "L6" scFvs), anti-CTLA-4 (CD152) scFvs (for example, "10A8" scFvs), anti-human CD28 scFvs (for  
10 example, "2E12" scFvs), anti-murine CD3 scFvs (for example, "500A2" scFvs), anti-human CD3 scFvs (for example, G19-4 scFvs), anti-murine 4-1BB scFvs (for example, "1D8" scFvs), anti-human 4-1BB scFvs (for example, "5B9" scFvs), anti-human CD45RO (for example, "UCHL-1" scFvs), and anti-human CD16 (for example, "Fc2" scFvs).

scFvs useful in the invention also include scFvs, including chimeric and  
15 humanized scFvs, having one or more amino acid substitutions. A preferred amino acid substitution is at amino acid position 11 in the variable heavy chain (the  $V_H$ ). Such a substitution may be referred to herein as "Xxx $V_H$ 11Zxx". Thus, for example, where the normally occurring amino acid at position  $V_H$ 11 is a Leucine, and a Serine amino acid residue is substituted therefor, the substitution is identified as "L  $V_H$ 11S" or "Leu  $V_H$ 11Ser."  
20 Other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residue normally found at position  $V_H$ 11 is deleted. Still other preferred embodiments of the invention include molecules containing scFvs wherein the amino acid residues normally found at positions  $V_H$ 10 and/or  $V_H$ 11 and/or  $V_H$ 12 are substituted or deleted.

25 Other binding regions within the molecules of the invention may include domains that comprise sites for glycosylation, for example, covalent attachment of carbohydrate moieties such as monosaccharides or oligosaccharides.

Still other binding regions within molecules of the invention include polypeptides that may comprise proteins or portions thereof that retain the ability to  
30 specifically bind another molecule, including an antigen. Thus, binding regions may

comprise or be derived from hormones, cytokines, chemokines, and the like; cell surface or soluble receptors for such polypeptide ligands; lectins; intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like; histocompatibility antigens; and so on. Binding regions derived from such molecules generally will include those portions of the molecules necessary or desired for binding to a target.

Certain constructs include binding regions that comprise receptor or receptor-binding domains. Receptor domains useful for binding to a target include, for example, a CD154 extracellular domain, or a CTLA-4 extracellular domain. In another example, the binding domain may include a first portion comprising, consisting essentially or, or consisting of, a CD154 extracellular domain and a second portion comprising, consisting essentially or, or consisting of, at least one immunoglobulin variable region polypeptide, said second portion including, for example, an scFv or a V<sub>H</sub>. Examples of other cell surface receptors that may comprise, consist essentially or, or consist of, or a portion of which may provide, a binding region or binding domain polypeptide, include, for example, HER1, HER2, HER3, HER4, epidermal growth factor receptor (EGFR), vascular endothelial cell growth factor, vascular endothelial cell growth factor receptor, insulin-like growth factor-I, insulin-like growth factor-II, transferrin receptor, estrogen receptor, progesterone receptor, follicle stimulating hormone receptor (FSH-R), retinoic acid receptor, MUC-1, NY-ESO-1, Melan-A/MART-1, tyrosinase, Gp-100, MAGE, BAGE, GAGE, any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene, carcinoembryonic antigen (CEA), and PyLT. Additional cell surface receptors that may be sources of binding regions or binding domain polypeptides include, for example, CD2, 4-1BB, 4-1BB ligand, CD5, CD10, CD27, CD28, CD152/CTLA-4, CD40, interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-4 (IL-4), interleukin-17 (IL-17) and interleukin-17 receptor (IL-17R). Still other cell surface receptors that may be sources of binding regions and/or binding domain polypeptides include, for example, CD59, CD48, CD58/LFA-3, CD72, CD70, CD80/B7.1, CD86/B7.2, B7-H1/B7-DC, IL-17, CD43, ICOS, CD3 (*e.g.*, gamma subunit, epsilon subunit, delta subunit), CD4, CD25, CD8, CD11b, CD14, CD56, CD69 and VLA-4 ( $\alpha_4\beta_7$ ). The following cell surface receptors are typically associated with B cells: CD19, CD20,

CD22, CD30, CD153 (CD30 ligand), CD37, CD50 (ICAM-3), CD106 (VCAM-1), CD54 (ICAM-1), interleukin-12, CD134 (OX40), CD137 (41BB), CD83, and DEC-205. These lists are not exhaustive. Binding regions such as those set forth above may be connected, for example, by a native or engineered IgD hinge region polypeptide, preferably a human or humanized native or engineered IgD hinge region polypeptide. The invention thus further provides constructs that comprise, consist essentially of, or consist of, two binding regions, for example, an scFv and a cell surface receptor (or portion thereof), connected by a third molecule, for example, an IgD hinge region polypeptide as described herein.

Various molecules of the invention described and claimed herein include a connecting region joining one end of the molecule to another end. Such connecting regions may comprise, for example, immunoglobulin hinge region polypeptides, including any hinge peptide or polypeptide that occurs naturally. A connecting region may also include, for example, any artificial peptide or other molecule (including, for example, non-peptide molecules, partial peptide molecules, and peptidomimetics, *etc.*) useful for joining the tail region and the binding region. These may include, for example, alterations of molecules situated in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions. Naturally occurring hinge regions include those located between the constant region domains, CH1 and CH2, of an immunoglobulin. Useful immunoglobulin hinge region polypeptides include, for example, human immunoglobulin hinge region polypeptides and llama or other camelid immunoglobulin hinge region polypeptides. Other useful immunoglobulin hinge region polypeptides include, for example, nurse shark and spotted ratfish immunoglobulin hinge region polypeptides. Human immunoglobulin hinge region polypeptides include, for example, wild type IgG hinges including wild-type human IgG1 hinges, human IgG-derived immunoglobulin hinge region polypeptides, a portion of a human IgG hinge or IgG-derived immunoglobulin hinge region, wild-type human IgA hinge region polypeptides, human IgA-derived immunoglobulin hinge region polypeptides, a portion of a human IgA hinge region polypeptide or IgA-derived immunoglobulin hinge region polypeptide, wild-type human IgD hinge region polypeptides, human Ig-D derived immunoglobulin hinge region polypeptides, a portion of a human IgD hinge region

polypeptide or IgD-derived immunoglobulin hinge region polypeptide, wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptides (which generally have 5 cysteine residues), human IgE-derived immunoglobulin hinge region polypeptides, a portion of a human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide or IgE-derived immunoglobulin hinge region polypeptide, and so on. A polypeptide “derived from” or that is “a portion or fragment of” an immunoglobulin polypeptide chain region regarded as having hinge function has one or more amino acids in peptide linkage, for example 15-115 amino acids, preferably 95-110, 80-94, 60-80, or 5-65 amino acids, preferably 10-50, more preferably 15-35, still more preferably 18-32, still more preferably 20-30, still more preferably 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids. Llama immunoglobulin hinge region polypeptides include, for example, an IgG1 llama hinge. The connecting region may comprise a stretch of consecutive amino acids from an immunoglobulin hinge region. For example, the connecting region can comprise at least five consecutive hinge region amino acids, at least ten consecutive hinge region amino acids, at least fifteen consecutive hinge region amino acids, at least 20 consecutive hinge region amino acids, and at least twenty five or more consecutive hinge region amino acids from human IgG hinge, human IgA hinge, human IgE hinge, camelid hinge region, IgG1 llama hinge region, nurse shark hinge region, and spotted ratfish hinge region, including for example an IgG<sub>1</sub> hinge region, a IgG<sub>2</sub> hinge region, a IgG<sub>3</sub> hinge region, an IgG<sub>3</sub> hinge region, and an IgG<sub>4</sub> hinge region.

Such connecting regions also include, for example, mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides. A mutated or otherwise altered or engineered immunoglobulin hinge region polypeptide may comprise, consist essentially of, or consist of, a hinge region that has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is the same or different from that of any included native or engineered CH2 and CH3 domains. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains one or more cysteine residues, for example, a wild-type human IgG or IgA hinge region that naturally comprises three cysteines. In such polypeptides the number of cysteine residues may be reduced by amino acid substitution or deletion or truncation, for

example. These polypeptides include, for example, mutated human or other IgG1 or IgG4 hinge region polypeptides containing zero, one, or two cysteine residues, and mutated human or other IgA1 or IgA2 hinge region polypeptides that contain zero, one, or two cysteine residues. Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, a wild-type immunoglobulin hinge region that contains three or more cysteine residues, for example, a wild-type human IgG2 hinge region (which has 4 cysteines) or IgG4 hinge region (which has 11 cysteines). Mutated or otherwise altered or engineered immunoglobulin hinge region polypeptides include those derived or constructed from, for example, an IgE CH2 wild-type immunoglobulin region that generally contains five cysteine residues. In such polypeptides the number of cysteine residues may be reduced by one or more cysteine residues by amino acid substitution or deletion or truncation, for example. Also included are an altered hinge region polypeptides in which cysteine residues in the hinge region are substituted with serine or one or more other amino acids that are less polar, less hydrophobic, more hydrophilic, and/or neutral. Such mutated immunoglobulin hinge region polypeptides include, for example, mutated hinge region polypeptides that contain one cysteine residue and that are derived from a wild-type immunoglobulin hinge region polypeptide having two or more cysteine residues, such as a mutated human IgG or IgA hinge region polypeptide that contains one cysteine residue and that is derived from a wild-type human IgG or IgA region polypeptide. Connecting region polypeptides include immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Mutated immunoglobulin hinge region polypeptides also include mutated hinge region polypeptides that exhibit a reduced ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide, and mutated hinge region polypeptides that allow expression of a mixture of monomeric and dimeric molecules. Mutated immunoglobulin hinge region polypeptides also include hinge region polypeptides engineered to contain a glycosylation site. Glycosylation sites include, for example, an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site, and a phosphoglycation site.

Specific connecting regions useful in molecules of the invention described and claimed herein include, for example, the following 18 amino acid sequences, DQEPKSCDKTHTCPPCPA, DQEPKSSDKTHTSPPPSPA, and DLEPKSCDKTHTCPPCPA. Other specific connecting regions include, for example, the mutant hinges within the sequences referred to herein as “2H7 scFv (SSS-S)H WCH2 WCH3” and “2H7 scFv (CSS)H WCH2 WCH3”, and the human IgA-derived hinge referred to herein as “2H7 scFv IgAH WCH2 WCH3”.

Tail regions within the molecules of the invention may include heavy chain constant region immunoglobulin sequences. Tail regions may thus include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide. At least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH2 and/or CH3 polypeptides may be derived from human IgG, human IgA, or human IgD molecules. Tail regions may also include, for example, a polypeptide having at least one of an immunoglobulin heavy chain CH3 constant region polypeptide and an immunoglobulin heavy chain CH4 constant region polypeptide. At least one of the immunoglobulin heavy chain CH3 constant region polypeptide and the immunoglobulin heavy chain CH4 constant region polypeptide may be derived from a human immunoglobulin heavy chain. Thus, for example, CH3 and/or CH4 polypeptides may be derived from human IgE. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. Additionally, both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgG1, IgG2, IgG3 and/or IgG4 subclasses. In other molecules of the invention at least one of the immunoglobulin heavy chain constant region polypeptides selected from a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region



polypeptide. An immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses. An immunoglobulin heavy chain CH3 region polypeptide included within a molecule of the invention may also, for example, be from the IgA1 and/or IgA2 subclasses. Additionally,

5 both the immunoglobulin heavy chain CH2 region polypeptide and the immunoglobulin heavy chain CH2 region polypeptide included within a molecule of the invention may, for example, be from the IgA1 and/or IgA2 subclasses. In still other molecules of the invention, the tail region may comprise or consist essentially of a CH2 and/or CH3 constant region polypeptide comprising a polypeptide from human IgA and/or human IgE. In other

10 embodiments, for example, the tail region within a molecule of the invention may include an immunoglobulin heavy chain CH2 and/or CH3 constant region polypeptide that is a mutated (for example, a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain in which, for example, the IgA CH3 constant region polypeptide is of human origin). The tail region may also comprise, consist essentially of, or consist of an

15 extracellular portion of a protein from the TNF superfamily, for example, CD154.

For molecules of the invention intended for use in humans, these regions will typically be substantially or completely human to minimize a potential human immune responses against the molecules and to provide appropriate effector functions. In certain embodiments of the invention, for example, the tail region includes a human IgG1 CH3

20 region sequence, a wild-type IgA heavy chain constant region polypeptide sequence that is capable or incapable of associating with J chain.

In preferred embodiments of the invention, a CH1 domain is not included in the tail region of the molecule, and the carboxyl end of the binding region is joined to the amino terminus of a CH2 portion of a tail region either directly or indirectly. A binding

25 region may be indirectly joined to a tail region, for example via a connecting region polypeptide or other connecting molecule.

The invention also includes molecules that have mutated CH2 and/or CH3 sequences within a tail region. For example, a molecule of the invention may include a mutated Fc domain that has one or more mutations introduced into the CH2, CH3 and/or

30 CH4 domains. In certain embodiments of the invention, molecules may include an IgA CH3

constant region polypeptide such as a human IgA CH3 constant region polypeptide in which two or more residues from the C-terminus have been deleted to yield a truncated CH3 constant region polypeptide. In other embodiments of the invention, molecules include a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain that comprises a C-terminal deletion of either four or 18 amino acids. However, the invention need not be so limited, such that molecules containing the mutated IgA CH3 constant region polypeptide may comprise a deletion of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30 or more amino acids, so long as the fusion protein is capable of specifically binding an antigen and capable of at least one immunological activity such as ADCC, CDC or complement fixation. The invention also includes molecules containing a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents interchain disulfide bond formation.

Various molecules of the invention include, for example, a binding domain scFv- fusion protein having a binding domain polypeptide comprising, consisting essentially of, or consisting of, (a) at least one immunoglobulin light chain variable region polypeptide, (b) at least one immunoglobulin heavy chain variable region polypeptide, and at least one linker peptide that joins the polypeptide of (a) and the polypeptide of (b). Such polypeptides may, for example, be derived from human immunoglobulins or non-human immunoglobulins.

Thus, in one aspect, the invention includes a non-naturally occurring single chain protein and/or  $V_H$  protein and/or  $V_L$  protein, or a desired portion of any of the above, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a flexible or other desired linker attached to said first polypeptide, a third polypeptide comprising a tail region, for example, an N-terminally truncated immunoglobulin heavy chain constant region polypeptide (or desired portion thereof) attached to the second polypeptide. The flexible linker may comprise, consist essentially of, or consist of, an immunoglobulin hinge region or portion thereof that has been mutated or otherwise altered or engineered, for example, one that

contains a number of cysteine residues that is less than the number of cysteine residues present in the wild type immunoglobulin hinge region or portion (for example, zero, one, or two cysteines in the case of IgG1 or IgG4), and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity, for example, ADCC, CDC, and/or complement fixation. This protein may include a binding domain polypeptide which is a single chain Fv. Additionally, this protein may include a binding domain polypeptide which is a single chain Fv wherein the heavy chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112. The protein may also include a binding domain polypeptide which is a single chain Fv wherein the light chain variable region of the single chain Fv has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

In another aspect, the invention includes a non-naturally occurring  $V_H$  protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule or construct, a  $V_H$  region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said  $V_H$  region. Amino acids may be substituted with either naturally-occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of  $V_H$  proteins, or desired portions thereof, that comprise, consist essentially of, or consist of, alone or in combination with any other molecule or construct, a  $V_H$  region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112 of said  $V_H$  region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a non-naturally occurring  $V_L$  protein, or a desired portion thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a  $V_L$  region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said  $V_L$  region. Amino acids may be substituted with either naturally-occurring or non-naturally occurring amino acids, or any other desired useful molecule.

Also described and claimed are uses of  $V_L$  proteins, or desired portions thereof, that comprises, consists essentially of, or consists of, alone or in combination with any other molecule, a  $V_L$  region or portion thereof that has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107 of said  
5  $V_L$  region. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

In yet another aspect, the invention includes a molecule comprising, consisting essentially of, or consisting of, (1) a  $V_H$  protein, or a desired portion thereof, wherein the  $V_H$  protein or portion thereof has an amino acid deletion or substitution at one or  
10 more of amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring  $V_L$  protein, or a desired portion thereof, alone or in combination with any other molecule, wherein the  $V_L$  protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Amino acids may be substituted with either naturally-occurring or non-naturally occurring  
15 amino acids, or any other desired useful molecule.

Also described and claimed are uses of a molecule comprising, consisting essentially of, or consisting of, (1) a  $V_H$  protein, or a desired portion thereof, wherein the  $V_H$  protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, and 112, and (2) a non-naturally occurring  $V_L$  protein,  
20 or a desired portion thereof, alone or in combination with any other molecule, wherein the  $V_L$  protein or portion thereof has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. Such uses include uses in phage display, yeast display, and ribosome display systems and methods.

The invention also includes molecular constructs wherein the binding domain  
25 is a single chain Fv and the heavy chain variable region of said single chain Fv has an amino acid substitution at amino acid position 11. The amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region may be selected from the group consisting of serine, threonine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. The invention thus includes, for example, a construct  
30 wherein the binding domain is a single chain Fv and the heavy chain variable region of said

single chain Fv has a serine amino acid substitution at amino acid position 11. Other amino acid position changes, substitutions, and deletions, are noted herein.

The invention also includes, for example, a construct wherein the binding domain is a single chain Fv and the amino acid at position 10 and/or 11 of the heavy chain  
5 variable region of said single chain Fv has been deleted.

In another aspect, the invention includes constructs wherein the binding region binds to a tumor or tumor-associated antigen. The binding region of a construct of the invention may bind, for example, to a cancer cell antigen. Cancer cell antigens to which constructs of the invention bind include cancer cell surface antigens and intracellular cancer  
10 cell antigens.

In yet another aspect, the invention includes a construct wherein the binding region binds to an antigen on an immune effector cell.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell antigen including, for example, a B cell antigen selected from the  
15 group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86. Constructs of the invention that bind to such B cell antigens include, for example, binding regions comprising an single chain Fv. Examples of such single chain Fv binding regions include molecules comprising or consisting essentially of single chain Fvs selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single  
20 chain Fv. Other examples include a binding region comprising, consisting essentially of, or consisting of, an extracellular domain of CTLA-4.

In another aspect, the invention includes a construct wherein the binding region binds to a B cell differentiation antigen. B cell differentiation antigens include, for example, CD19, CD20, CD21, CD22, CD23, CD37, CD40, CD45RO, CD80, CD86, and  
25 HLA class II.

In another aspect, the invention includes a construct wherein the binding region binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86,

CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

5       The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of existing in solution as a monomer or in substantially monomeric form.

      The invention also includes protein constructs having a binding region, a tail region, and a connecting region, wherein the protein construct is capable of forming a complex comprising two or more of said protein constructs including, for example, wherein said complex is a dimer.

10       In another aspect, constructs of the invention are capable of participating in or inducing or eliciting or helping to induce or elicit, directly or indirectly, at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity (or complement-mediated lysis), complement fixation, induction of apoptosis, induction of one or more biologically active  
15       signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

      In another aspect, binding constructs of the invention are capable of induction of biologically active signals by activation or inhibition of one or more molecules selected  
20       from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components. Such biologically active molecules are, for example, proteases. Other biologically active molecules are, for example, cytokines, including by way of example monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and  
25       interleukins.

      In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of one or more immune effector cells that results in antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

5 In another aspect, constructs of the invention are capable of participating in and/or initiating apoptosis within target cells, for example, by activating one or more signalling mechanisms or molecules.

In another aspect, constructs of the invention are capable of induction, or participation in the induction, of cellular activation, wherein said activation leads to changes  
10 in cellular transcriptional activity. In one embodiment, cellular transcriptional activity is increased. In another embodiment, cellular transcriptional activity is decreased.

In another aspect, constructs of the invention having tail regions comprising, consisting essentially of, or consisting of, constant regions from IgA or IgE molecules, are capable of induction, or participation in the induction, of degranulation of neutrophils and/or  
15 mast cells.

In another aspect, constructs of the invention are capable of promotion, or participation in the promotion, of neutralization of an infectious agent, wherein said infectious agent is, for example, a bacterium, a virus, a parasite, or a fungus.

In another aspect, constructs of the invention are capable of promoting, or  
20 participating in the promotion of, neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins. Such toxins include, for example, exotoxins selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin,  
25 and *Bacillus anthracis* EF toxin. Other toxins include, for example, saxitoxins, tetrodotoxin, mushroom toxins (amatoxins, gyromitrin, orellanine, *etc.*), aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

In another aspect, constructs of the invention are capable of binding to an intracellular target to, for example, effect (or participate in effecting) a cellular function.  
30 Such constructs include, for example, constructs that include a tail region comprising,

consisting essentially of, or consisting of, a native or engineered IgA CH2 domain region and a native or engineered IgA CH3 domain region, said tail region being capable of binding J chain. Such a tail region is found, for example, in the 2H7 scFv IgAH WlgACH2 WCH3 + JChain construct. Thus, the invention includes constructs having, for example, an  
5 "Anti-Intracellular Target" binding domain (for example, and "Anti-Intracellular Target" scFv), a connecting region, and a native or engineered IgA constant region capable of binding J chain (for example, WlgACH2 WCH3).

In still another aspect, constructs of the invention include a molecule wherein an N-terminally immunoglobulin heavy chain constant region polypeptide comprises an IgG  
10 CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

In yet another aspect, the invention includes a method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein molecule that is less than about 120kK, or less than about  
15 150kD, as measured, for example, by HPLC and non-reducing gels and consists essentially of (a) a first protein or peptide molecule that is capable of binding to cells within said target cell population, and (b) a second protein or peptide molecule that is capable of (i) binding to an Fc receptor and/or (ii) inducing target cell apoptosis, and/or (iii) fixing complement, wherein said first protein or peptide molecule is directly connected to said second protein or  
20 peptide molecule, or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

Various specific constructs of the invention include, by way of example only,  
25 the following:

1. 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3
2. 2H7 scFv VH L11S IgE CH2 CH3 CH4
3. 2H7 scFv VH L11S mIgE CH2 CH3 CH4
4. 2H7 scFv VH L11S mIgAH WlgACH2 T4CH3
- 30 5. 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3



	6. 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3
	7. 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3
	8. 2HU scFv VH L11S (CSS-S) H P331S CH2 WCH3
	9. 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3
5	10. 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3
	11. 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3
	12. 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3
	13. G28-1 scFv (SSS-S) H WCH2 WCH3
	14. G28-1 scFv IgAH WCH2 WCH3
10	15. G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3
	16. G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3
	17. G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3
	18. G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3
	19. CTLA4 (SSS-S) H P238SCH2 WCH32
15	20. CTLA4 (CCC-P) WH WCH2 WCH3
	21. FC2-2 scFv (SSS-S) H WCH2 WCH3
	22. FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3
	23. UCHL-1 scFv (SSS-S) H WCH2 WCH3
	24. UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3
20	25. 5B9 scFv (SSS-S) H WCH2 WCH3
	26. 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3
	27. 2H7 scFv (SSS-S) H WCH2 WCH3
	28. 2H7 scFv (SSS-S) H P238SCH2 WCH3
	29. 2H7 scFv IgAH WCH2 WCH3
25	30. 2H7 scFv IgAH WlgACH2 T4CH3
	31. 2H7 scFv IgAH WlgACH2 WCH3 + JChain
	32. 2H7 scFv (CCC-P) WH WCH2 WCH3
	33. 2H7 scFv (SSS-S) H WCH2 F405YCH3
	34. 2H7 scFv (SSS-S) H WCH2 F405ACH3
30	35. 2H7 scFv (SSS-S) H WCH2 Y407ACH3

	36. 2H4 scFv (SSS-S) HWCH2 F405A, Y407ACH3
	37. 2H7 <sub>scFv</sub> (CSS-S) H WCH2 WCH3
	38. 2H7 scFv (SCS-S) H WCH2 WCH3
	39. 2H7 scFv (SSC-P) H WCH2 WCH3
5	40. 2H7 scFv (CSC-S) H WCH2 WCH3
	41. 2H7 scFv (CCS-P) H WCH2 WCH3
	42. 2H7 scFv (SCC-P) H WCH2 WCH3
	43. 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3
	44. 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3
10	45. G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3
	46. G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3
	47. G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3
	48. G28-1 scFv VH L11S mlgE CH2 CH3 CH4
	49. G28-1 scFv VH L11S mIgAH WlgACH2 T4CH3
15	50. G28-1 scFv VH L11S hlgE CH2 CH3 CH4
	51. G28-1 scFv VH L11S hIgAH WlgACH2 T4CH3
	52. HD37 scFv IgAH WCH2 WCH3
	53. HD37 scFv (SSS-S) H WCH2 WCH3
	54. HD37 scFv VH L11S (SSS-S) H WCH2 WCH3
20	55. L6 scFv IgAH WCH2 WCH3
	56. L6 scFv VHL11S (SSS-S) H WCH2 WCH3
	57. 2H7 scFv-llama IgG1
	58. 2H7 scFv-llama IgG2
	59. 2H7 scFv-llama IgG3
25	60. CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3
	61. CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3
	62. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	63. 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	64. 40.2.36 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
30	65. 2H7 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT

	66. G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	67. 2e12 scFv (SSS-s)H WCH2 WCH3—hCD80TM/CT
	68. 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT
	69. 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT
5	70. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT
	71. 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT
	72. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp3-TM/CT
	73. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT
	74. 2e12 scFv (SSS-s)H WCH2 WCH3—mcasp8--TM/CT
10	75. 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT
	76. 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp3--TM/CT
	77. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT
	78. 2e12 scFv (SSS-s)H WCH2 WCH3—hcasp8--TM/CT
	79. 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT
15	80. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT
	81. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hCD80TM/CT
	82. 1D8 scFv—mIgAT4—hCD80TM/CT
	83. 1D8 scFv--hIgE—hCD80TM/CT
	84. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-TM/CT
20	85. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT
	86. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp3-TM/CT
	87. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp3-TM/CT
	88. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasp8--TM/CT
	89. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasp8-TM/CT
25	90. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp3--TM/CT
	91. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp3-TM/CT
	92. 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasp8--TM/CT
	93. 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hcasp8-TM/CT L6 scFv (SSS-S) H WCH2 WCH3
30	94. 2H7 scFv CD154 (L2)

95. 2H7 scFv CD154 (S4)
96. CTLA4 IgAH IGACH2CH3
97. CTLA4 IgAH IgACH2 T4CH3
98. 2H7 scFv IgAH IgACH2CH3
- 5 99. 2H7 scFv IgAH IgAHCH2 T18CH3
100. 2H&-40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-cd20-  
anti-cd40)
101. 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT
102. G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT
- 10 103. 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT

These and other aspects of the present invention will become further apparent upon reference to the following detailed description and attached drawings. As noted herein, all referenced patents, articles, documents, and other materials disclosed or identified herein are hereby incorporated by reference in their entireties as if each was incorporated individually.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** shows DNA and deduced amino acid sequences [SEQ ID NOS:\_\_\_] of 2H7scFv-Ig, a binding domain-immunoglobulin fusion protein capable of specifically binding CD20.

**Figure 2** shows production levels of 2H7 scFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified 2H7 scFv-Ig to CHO cells expressing CD20.

**Figure 3** shows SDS-PAGE analysis of multiple preparations of isolated 2H7scFv-Ig protein.

**Figure 4** shows complement fixation (Fig. 4A) and mediation of antibody-dependent cellular cytotoxicity (Fig. 4B) by 2H7scFv-Ig.

**Figure 5** shows the effect of simultaneous ligation of CD20 and CD40 on growth of normal B cells.

**Figure 6** shows the effect of simultaneous ligation of CD20 and CD40 on CD95 expression and induction of apoptosis in a B lymphoblastoid cell line.

**Figure 7** shows DNA and deduced amino acid sequences [SEQ ID NOS:\_\_\_] of 2H7scFv-CD154 L2 (Fig. 7A, SEQ ID NOS:\_\_\_) and 2H7scFv-CD154 S4 (Fig. 7B, SEQ ID NOS:\_\_\_) binding domain-immunoglobulin fusion proteins capable of specifically binding CD20 and CD40.

**Figure 8** shows binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins to CD20+ CHO cells by flow immunocytometry.

**Figure 9** shows binding of Annexin V to B cell lines Ramos, BJAB, and T51 after binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein to cells.

**Figure 10** shows effects on proliferation of B cell line T51 following binding of 2H7scFv-CD154 binding domain-immunoglobulin fusion protein.

**Figure 11** depicts schematic representations of the structures of 2H7ScFv-Ig fusion proteins [SEQ ID NOS:\_\_\_] referred to as CytosB or CytosB derivatives: CytosB-MHWTG1C (2H7 ScFv, mutant hinge, wild-type human IgG1 Fc domain), CytosB-MHMG1C (2H7 ScFv, mutant hinge, mutated human IgG1 Fc domain) and CytosB-IgAHTWG1C (2H7 ScFv, human IgA-derived hinge [SEQ ID NO:\_\_\_], wild-type human IgG1 Fc domain). Arrows indicate position numbers of amino acid residues believed to contribute to FcR binding and ADCC activity (heavy arrows), and to complement fixation (light arrows). Note absence of interchain disulfide bonds.

**Figure 12** shows SDS-PAGE analysis of isolated CytosB and 2H7scFv-CD154 binding domain-immunoglobulin fusion proteins.

**Figure 13** shows antibody dependent cell-mediated cytotoxicity activity of CytosB derivatives.

**Figure 14** shows complement dependent cytotoxicity of CytosB derivatives.

**Figure 15** shows serum half-life determinations of CytosB-MHWTG1C in macaque blood samples.

**Figure 16** shows effects of CytosB-MHWTG1C on levels of circulating CD40+ B cells in macaque blood samples.

**Figure 17** shows production levels of HD37 (CD19-specific) ScFv-Ig by transfected mammalian cell lines and generation of a standard curve by binding of purified HD37 ScFv-Ig to cells expressing CD19.

**Figure 18** shows production levels of L6 (carcinoma antigen) ScFv-Ig by transfected, stable CHO lines and generation of a standard curve by binding of purified L6 ScFv-Ig to cells expressing L6 antigen.

**Figure 19** shows antibody dependent cell-mediated cytotoxicity activity of binding domain-immunoglobulin fusion proteins 2H7 ScFv-Ig, HD37 ScFv-Ig and G28-1 (CD37-specific) ScFv-Ig.

**Figure 20** shows antibody dependent cell-mediated cytotoxicity activity of L6 ScFv-Ig fusion proteins.

**Figure 21** shows SDS-PAGE analysis of L6 ScFv-Ig and 2H7 ScFv-Ig fusion proteins.

**Figure 22** shows SDS-PAGE analysis of G28-1 ScFv-Ig and HD37 ScFv-Ig fusion proteins.

**Figure 23** presents a sequence alignment of immunoglobulin hinge and CH2 domains of human IgG1 (SEQ ID NO:\_\_) with the hinge and CH2 domains of llama IgG1 (SEQ ID NO:\_\_), IgG2 (SEQ ID NO:\_\_), and IgG3 (SEQ ID NO:\_\_).

**Figure 24** illustrates migration of purified 2H7 scFv llama IgG fusion proteins in a 10% SDS polyacrylamide gel. Purified fusion proteins (5  $\mu$ g per sample) were prepared in non-reducing sample buffer (lanes 2-5) and in reducing sample buffer (lanes 6-9). Lane 1: molecular weight markers (non-reduced); lanes 2 and 6: 2H7 scFv-llama IgG1 (SEQ ID NO:\_\_); Lanes 3 and 7: 2H7 scFv-llama IgG2 (SEQ ID NO:\_\_); lanes 4 and 8: 2H7 scFv-llama IgG3 (SEQ ID NO:\_\_); and Lanes 5 and 9: Rituximab (chimeric anti-CD20 antibody (human IgG1 constant region)).

**Figure 25** shows binding of 2H7 scFv-llama IgG1 (SEQ ID NO:\_\_), 2H7 scFv-llama IgG2 (SEQ ID NO:\_\_), and 2H7 scFv-llama IgG3 (SEQ ID NO:\_\_) to CD20+ CHO cells detected by flow immunocytometry.

**Figure 26** depicts CDC activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:\_\_), 2H7 scFv-llama IgG2 (SEQ ID NO:\_\_), and 2H7 scFv-

llama IgG3 (SEQ ID NO:\_\_), and 2H7 scFv human IgG1 (2H7 scFv IgG WTH WTCH2CH3) (SEQ ID NO:\_\_) against BJAB cells in the presence of rabbit complement. Rituximab was included as a control.

**Figure 27** shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:\_\_), 2H7 scFv-llama IgG2 (SEQ ID NO:\_\_), and 2H7 scFv-llama IgG3 (SEQ ID NO:\_\_). Effector cells (human PBMC) were combined with target cells (BJAB cells) at three different ratios, 1:25, 1:50, and 1:100. Rituximab was included as a control. Each data point represents three separate measurements.

**Figure 28** shows antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv llama IgG fusion proteins, 2H7 scFv-llama IgG1 (SEQ ID NO:\_\_), 2H7 scFv-llama IgG2 (SEQ ID NO:\_\_), and 2H7 scFv-llama IgG3 (SEQ ID NO:\_\_). Effector cells (llama PBMC) were combined with target cells (BJAB cells) at three different ratios, 1:25, 1:50, and 1:100. Rituximab was included as a control. Each data point represents three separate measurements.

**Figure 29** depicts complement dependent cytotoxicity activity of Reh cells (acute lymphocytic leukemia) expressing scFv-Ig fusion proteins on the cell surface. Reh cells were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains. Complement dependent cytotoxicity activity was measured in the presence and absence of rabbit complement (plus C' and no C', respectively). The data represent the average of duplicate samples. Reh anti-hCD152 scFvIg: Reh cells transfected with polynucleotide 10A8 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:\_\_); Reh anti-hCD28scFvIg: 2E12 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:\_\_); Reh anti-hCD40scFvIg: 4.2.220 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:\_\_); and Reh anti-hCD20scFvIg: 2H7 scFv IgG MTH (SSS) MT CH2CH3 (SEQ ID NO:\_\_).

**Figure 30** presents antibody dependent cell-mediated cytotoxicity activity of Reh cells that were transfected with constructs encoding scFv antibodies specific for human costimulatory molecules, CD152, CD28, CD40, and CD20, as described for Figure 29, and

for murine CD3, fused to human mutant IgG1 hinge and mutant CH2 and wild type CH3 (Reh anti-mCD3scFv designating Reh cells transfected with polynucleotide 500A2 scFv IgG MTH (SSS) MTCH2WTCH3 SEQ ID NO:\_\_), which was fused to human CD80 transmembrane and cytoplasmic tail domains. The data represent the average of quadruplicate samples.

**Figure 31** lists immunoglobulin constant region constructs that were used in experiments illustrated in subsequent figures.

**Figure 32** depicts complement dependent cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_) (2  $\mu$ g/ml) and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO:\_\_) (2  $\mu$ g/ml), in the presence and absence of rabbit complement (plus C' and no C', respectively). The target cells were Reh cells and Reh cells transfected with CD80 (Reh CD80.10).

**Figure 33** shows antibody dependent cell-mediated cytotoxicity activity of CTLA-4 Ig fusion proteins, CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_) (2  $\mu$ g/ml) and CTLA-4 IgG MTH MTCH2WTCH3 (SEQ ID NO:\_\_) (2  $\mu$ g/ml). Effector cells, human PBMC, were added to target cells, Reh or Reh CD80.1, at the ratios indicated. Figure 33A presents the level of natural killing in Reh CD80.1 cells in the absence of any Ig fusion protein. Figure 33B presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG MTH MTCH2WTCH3, and Figure 33C presents antibody dependent cell-mediated cytotoxicity mediated by CTLA-4 IgG WTH (CCC) WTCH2CH3. Each data point represents the average percent specific killing measured in four sample wells.

**Figure 34** illustrates binding of 2H7 (anti-CD20) scFv Ig fusion proteins to (CD20+) CHO cells by flow immunocytometry.

**Figure 35** presents an immunoblot of 2H7 scFv IgG and IgA fusion proteins. COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. The expressed polypeptides were immune precipitated with protein A, separated in a non-reducing SDS polyacrylamide gel, and then transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lane 1: vector only; lane 2: 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_); lane 3: 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:\_\_); lane 4: 2H7



scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:\_\_); lane 5: 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO:\_\_); and lane 6: 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:\_\_).

**Figure 36** illustrates binding of 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO:\_\_) and 2H7 scFv IgAH IgAT4 (SEQ ID NO:\_\_) to (CD20+) CHO cells by flow immunocytofluorimetry. The source of the polypeptides was culture supernatants from transiently transfected COS cells. COS cells transfected with a plasmid comprising a sequence encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid containing nucleotide sequence encoding human J chain.

**Figure 37** illustrates antibody dependent cell-mediated cytotoxicity activity of anti-CD20 (2H7) scFv Ig fusion proteins against BJAB target cells using whole blood as the source of effector cells. Purified 2H7 scFv Ig fusion proteins were titrated and combined with <sup>51</sup>Cr-labeled BJAB cells ( $5 \times 10^4$ ) and whole blood (1:4 final dilution). Each data point represents the average percent specific killing measured in four sample wells.

**Figure 38** demonstrates antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv Ig fusion proteins ( $5 \mu\text{g/ml}$ ) against <sup>51</sup>Cr-labeled BJAB cells at 0.25, 0.125, and 0.625 dilutions of whole blood. Each data point represents the average percent specific killing measured in four sample wells.

**Figure 39** shows a comparison of antibody dependent cell-mediated cytotoxicity activity of 2H7 scFv IgG MTH (SSS) WTCH2CH3 ( $5 \mu\text{g/ml}$ ) and 2H7 scFv IgAH IgACH2CH3 ( $5 \mu\text{g/ml}$ ) when human PBMC are the source of effector cells (Figure 39A) and when human whole blood is the source of effector cells (Figure 39B).

**Figure 40** presents an immunoblot of 2H7 scFv IgG fusion proteins. COS cells were transiently transfected with various 2H7 scFv Ig fusion protein constructs. Culture supernatants containing the expressed polypeptides were separated in a non-reducing SDS polyacrylamide gel, and then were transferred to a polyvinyl fluoride membrane. Proteins were detected using an anti-human IgG (Fc specific) horseradish peroxidase conjugate. Lanes 1-5: purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 at 40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng per lane, respectively. Culture supernatants were separated in lanes 6-9. Lane 6: 2H7 scFv IgG WTH (CCC) WTCH2CH3; lane 7: 2H7 scFv IgG MTH

(CSS) WTCH2CH3; lane 8: 2H7 scFv IgG MTH (SCS) WTCH2CH3; and lane 9: 2H7 scFv VHSE11 IgG MTH (SSS) WTCH2CH3. The molecular weight (kDa) of marker proteins is indicated on the left side of the immunoblot.

**Figure 41** illustrates cell surface expression of 1D8 (anti-murine 4-1BB) scFv IgG WTH WTCH2CH3-CD80 fusion protein on K1735 melanoma cells by flow immunofluorimetry (Fig. 41A). The scFv fusion protein was detected with phycoerythrin-conjugated F(ab')<sub>2</sub> goat anti-human IgG. Fig. 41B depicts growth of tumors in naïve C3H mice transplanted by subcutaneous injection with wild type K1735 melanoma cells (K1735-WT) or with K1735 cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 (K1735-1D8). Tumor growth was monitored by measuring the size of the tumor. Fig. 41C demonstrates the kinetics of tumor growth in naïve C3H mice injected intraperitoneally with monoclonal antibodies to remove CD8<sup>+</sup>, CD4<sup>+</sup>, or both CD4<sup>+</sup> and CD8<sup>+</sup> T cells prior to transplantation of the animals with K1735-1D8 cells.

**Figure 42** demonstrates therapy of established K1735-WT tumors using K1735-1D8 as an immunogen. Six days after mice were transplanted with K1735-WT tumors, one group (five animals) was injected subcutaneously with K1735-1D8 cells (open circles) or irradiated K1735-WT cells (solid squares) on the contralateral side. A control group of mice received PBS (open squares). Treatments were repeated on the days indicated by the arrows.

**Figure 43** shows the growth of tumors in animals that were injected subcutaneously with  $2 \times 10^6$  K1735-WT cells (solid squares) and the growth of tumors in animals that were injected subcutaneously with  $2 \times 10^6$  K1735-WT cells plus  $2 \times 10^5$  K1735-1D8 cells (open triangles).

**Figure 44** presents a flow cytometry analysis of antigen104 murine sarcoma tumor cells transfected with 1D8 scFv IgG WTH WTCH2CH3-CD80 isolated after repeated rounds of panning against anti-human IgG. Transfected cells expressing 1D8 scFv IgG WTH WTCH2CH3-CD80 were detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (depicted in black). Untransfected cells are shown in gray.

**Figure 45** illustrates migration of various 2H7 scFv Ig fusion proteins in a 10% SDS-PAGE gel. 2H7 was the anti-CD20 scFv and 40.2.220 was the anti-CD40 scFv.

Lane 1: Bio-Rad prestained molecular weight standards; lane 2: anti-CD20 scFv IgG MTH (SSS) MTCH2WTCH3; lane 3: anti-CD20 scFv IgG MTH (SSS) WTCH2CH3; lane 4: 2H7 scFv IgAH IgG WTCH2CH3; lane 5: anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3; lane 6: Rituximab; lane 7: Novex Multimark® molecular weight standards.

**Figure 46** illustrates effector function as measured in an antibody dependent cell-mediated cytotoxicity assay of 2H7 Ig fusion proteins that contain a mutant CH2 domain or wild type CH2 domain. The percent specific killing of BJAB target cells in the presence of human PBMC effector cells by 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (diamonds) was compared to 2H7 scFv IgG MTH (SSS) WTCH2CH3 (squares) and 2H7 scFv IgAH IgG WTCH2CH3 (triangles) and Rituximab (circles).

**Figure 47** shows cell surface expression of an anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO:\_\_) fusion protein on Reh cells (Fig. 47A) and T51 lymphoblastoid cells (Fig. 47B) by measuring the linear fluorescent equivalent (LFE) using flow immunocytofluorimetry.

**Figure 48** presents the percent specific killing of untransfected Reh and T51 cells and the percent specific killing of Reh cells (Reh anti-hCD3) (Fig. 48A) and T51 cells (T51 anti-hCD3) (Fig. 48B) that were transfected with a construct encoding scFv antibodies specific for human CD3, fused to human IgG1 wild-type hinge-CH2-CH3, which was fused to human CD80 transmembrane and cytoplasmic tail domains (anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 (SEQ ID NO:\_\_). Human PBMC (effector cells) were combined with BJAB target cells at the ratios indicated.

**Figure 49** illustrates binding of 5B9, an anti-murine CD137 (4-1BB) monoclonal antibody, and a 5B9 scFv IgG fusion protein (5B9 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:\_\_)) to stimulated human PBMC. Binding of the 5B9 scFv IgG fusion protein was detected by flow immunocytofluorimetry using FITC conjugated goat anti-human IgG. Binding of the 5B9 monoclonal antibody was detected with FITC conjugated goat anti-mouse IgG.

**Figure 50** illustrates the effect of the LV<sub>H</sub>11S mutation on the expression of 2H7 LV<sub>H</sub>11S scFv WCH2 WCH3 ("CytosB scFv Ig"; SEQ ID NO:\_\_) in CHO cell lines.

**Figure 51** shows a semi-quantitative SDS-PAGE analysis examining the expression of 2H7 LV<sub>H11S</sub> scFv WCH2 WCH3 (SEQ ID NO:\_\_) when transiently transfected in CHO cells. Lanes 2-5 are various amounts of 2H7 LV<sub>H11S</sub> scFv WCH2 WCH3. Lanes 6-10 are 10µl samples from five different clones expressing 2H7 LV<sub>H11S</sub> scFv WCH2 WCH3.

**Figure 52** shows differences in binding capacity between a G28-1 LV<sub>H11S</sub> scFv Ig construct (SEQ ID NO: \_\_) and a G28-1 wild type scFv Ig binding domain fusion protein construct (SEQ ID NO:\_\_), both obtained from transiently transfected COS cells. Binding to Ramos cells was determined using flow cytometry. The data illustrates a significant increase in binding of the V<sub>H11S</sub> protein to CD37+Ramos cells.

**Figure 53** illustrates increased levels of expression of a G28-1 LV<sub>H11S</sub> scFv Ig construct (SEQ ID NO: \_\_) compared to a G28-1 wild type scFv Ig construct in COS. Protein levels were compared using immunoblot analysis. Both immunoblot gels have quantitated amounts of purified a G28-1 scFv Ig (SSS-S) H WCH2 WCH3 construct of the invention in lanes 1-4. Lanes 5-9 of the first immunoblot represent five different clones each transfected with G28-1 scFv (SSS-S) H WCH2 WCH3, while lanes 5-9 of the second immunoblot represent five different clones transfected with G28-1 LV<sub>H11S</sub> scFv (SSS-S) H WCH2 WCH3. The immunoblots illustrate that the LV<sub>H11S</sub> form causes the G28-1 scFv Ig construct to express at very high levels.

**Figure 54** illustrates the binding of 2H7 scFv Ig derivatives with altered hinges (SEQ ID NOs: \_\_, \_\_, \_\_, \_\_, \_\_, \_\_, \_\_) to CHO cells expressing CD20 (CD20+ CHO) by flow cytometry, and indicates that these altered connecting region hinge constructs (including (SSS-S), (CSS-S), (SCS-S) and (CSC-S) hinge regions) retain binding function to CD20.

**Figure 55** shows the ability to mediate antibody dependent cell-mediated cytotoxicity of various constructs against Bjab targets: (A) 2H7 scFv Ig constructs of the invention that contain connecting regions comprising (CSS-S), (SCS-S), (CSC-S), and (SSS-S) hinges (SEQ ID NOs: \_\_, \_\_, \_\_, \_\_, \_\_, \_\_, \_\_) and (B) 2H7 scFv constructs of the invention with various connecting regions and tail regions (SEQ ID NOs: \_\_, \_\_, \_\_, \_\_, \_\_, \_\_).

Percent specific killing is compared to total killing induced by a detergent. The controls are

natural killing in target cells with effectors added and a 2H7 construct with an IgA hinge connecting region and IgA-derived tail region that does not bind PBMC effectors.

**Figure 56** illustrates the ability of various 2H7 scFv Ig constructs of the invention (SEQ ID NO: \_\_, \_\_, \_\_, \_\_, \_\_) that include connecting regions having various hinge regions (*e.g.*, (CSC-S), (SSS-S), (SCS-S), and (CSS-S)) to mediate complement activity in Ramos cells. Percent specific killing is measured against the control of complement only, and 100% killing was determined by exposure of cells to detergent.

**Figure 57** illustrates the shows the binding of 2H7 scFv Ig constructs of the invention containing different tail regions (SEQ ID NO: \_\_, \_\_, \_\_) to CD20+ CHO using immunocytofluorimetry. The different proteins were detected using FITC conjugated to anti-IgG, anti-IgA, and anti-IgE.

**Figure 58A** shows the binding of 2H7 V<sub>H</sub> L11S scFv IgE<sub>H</sub>2CH3CH4, purified using Hydrophobic charge induction chromatography (HCIC) and eluted at different pHs 4.0 and 3.5, (SEQ ID NO: \_\_) in CD20+ CHO cells by flow cytometry, indicating that the proteins bound CD20 whether eluted at pH 4.0 or 3.5. **Figure 58B** is a data graph indicating the ability of these 2H7 V<sub>H</sub> L11S scFv IgE constructs of the invention to mediate, for example, ADCC in Bjab target cells.

**Figure 59** shows the binding capacity of G28-1 V<sub>H</sub> L11S mIgE<sub>H</sub>2CH3CH4 (SEQ ID NO: \_\_) (A) to Bjab and Ramos target cells and (B) to CD20+ CHO cells by flow cytometry.

**Figure 60** shows the High Performance Liquid Chromatography (HPLC) profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H (P238S)CH2 WCH3 (SEQ ID NO: \_\_) (B) 2H7 scFv (CSS-S) H WCH2 WCH3, (SEQ ID NO: \_\_) (C) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO: \_\_) and (D) 2H7 scFv (SSS-S) H WCH2 (Y407A)CH3 (SEQ ID NO: \_\_), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B, C, and D. See Example 40.

**Figure 61** shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO: \_\_) (B) 2H7 scFv (CSC-S) H WCH2 WCH3, (SEQ ID NO: \_\_) (C) 2H7 scFv (CCC-P) H WCH2 WCH3, (SEQ ID

NO: \_\_) and (D) 2H7 scFv IgAH WCH2 WCH3 (SEQ ID NO: \_\_), indicating that construct A has apparent molecular weight forms of 100kD and 75kD and that, by introducing certain changes a predominant 75kD molecular weight form is obtained, as seen in constructs B and C, while construct D (which has an IgA tail region) has an apparent molecular weight of 150kD. See Example 40.

**Figure 62** shows the HPLC profiles of various protein constructs of the invention (A) 2H7 scFv (SSS-S) H WCH2 WCH3, (SEQ ID NO: \_\_) (B) 2H7 scFv (SCS-S) H WCH2 WCH3, (SEQ ID NO: \_\_) (C) 2H7 scFv IgA 3TCH2 WCH3, (SEQ ID NO: \_\_) and (D) 2H7 scFv (SSS-S) H WCH2 (F405A Y407A)CH3 (SEQ ID NO: \_\_), indicating that construct A has two forms with apparent molecular weights at 100kD and 75kD, construct B has a predominant form with an apparent molecular weight of 75kD, while construct C with a T4 mutation leads to three forms with apparent molecular weights near 600kD and construct D with a double point mutation in the CH3 region leads to a predominant form having an apparent molecular weight less than 44kD. A T4 mutation here refers to a truncation of four amino acids from a CH3 region. See Example 40.

**Figure 63** compares the effect on binding CD20+CHO cells by 2H7 V<sub>H</sub> L11S scFv Ig constructs (SEQ ID NOs: \_\_,\_\_), with and without F405A and Y407A alterations in the CH3 region, by flow cytometry, indicating a loss of binding capability with this double amino acid change. See Example 41.

**Figure 64** shows the binding capacity of FITC conjugated 2H7 V<sub>H</sub> L11S scFv Ig derivatives (SEQ ID NOs: \_\_,\_\_,\_\_,\_\_) to CH20+ CHO cells by flow cytometry, indicating that these constructs do not lose binding capacity when conjugated to a fluorescent marker. See Example 41.

**Figure 65** shows a nonreducing SDS-PAGE analysis examining 10 µg (per lane) of various purified 2H7 V<sub>H</sub> L11S scFv Ig constructs of the invention (SEQ ID NOs: \_\_,\_\_,\_\_,\_\_,\_\_,\_\_), indicating an apparent molecular weight for each construct in reference to a standard molecular weight marker in lane 1. See Example 41.

**Figure 66** compares the CH2 domain sequences of four different human IgG regions, hIgG1, hIgG2, hIgG3, hIgG4, and one rat region, rIgG2b. Point mutations affecting ADCC and CDC are labeled with arrows. See Example 52.

**Figure 67** demonstrates the ability of various 2H7 V<sub>H</sub> L11S scFv Ig constructs (SEQ ID NOs: \_\_,\_\_) to mediate ADCC in CHO and Lec13 CHO transiently transfected cells, indicating that constructs expressed in Lec 13 CHO cells had a 20% increase in specific killing over the same construct expressed in regular CHO cells. See Example 42.

**Figure 68** shows SDS-PAGE analysis, both reduced and nonreduced, of high and low affinity alleles of soluble CD 16(ED) (SSS-S) H P283S CH2 WCH3 (SEQ ID NOs: \_\_,\_\_). See Example 43.

**Figure 69** demonstrates the different binding capabilities of the high and low affinity CD16 fusion proteins (SEQ ID NOs: \_\_,\_\_) to 2H7 V<sub>H</sub> L11S scFv (CSC-S) WCH2 WCH3 (SEQ ID NO: \_\_) or 2H7 V<sub>H</sub> L11S scFv (SSS-S) WCH2 WCH3 (SEQ ID NO: \_\_), and indicates a loss of high and low affinity allele binding using P238S CH2 constructs. See Example 43.

**Figure 70** shows a diagram of (A) an assay used to detect changes in Fc receptor binding using FITC conjugated CD16 extracellular domain Ig fusion protein with a mutated tail, which eliminates self-association and B) a mammalian display system using cell surface expression of SMIPs. See Example 44.

**Figure 71** shows the induction of apoptosis in Bjab and Ramos cells by various mAbs (SEQ ID NOs: \_\_,\_\_,\_\_,\_\_,\_\_,\_\_,\_\_) and scFvIg constructs of the invention (SEQ ID NOs: \_\_,\_\_,\_\_,\_\_,\_\_,\_\_,\_\_). See Example 45.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel molecules useful, for example, as therapeutics, as well as for other purposes including diagnostic and research purposes. Such molecules have, for example, antigen-binding or other binding function(s) and, for example, one or more effector functions. The invention includes molecular constructs, including binding domain-immunoglobulin fusion proteins, and related compositions and methods, which will be useful in immunotherapeutic and immunodiagnostic applications, and in research methods, and which offer certain advantages over antigen-specific compounds and polypeptides of the prior art. The constructs, including fusion proteins, of the present

invention are preferably single polypeptide chains that comprise, in pertinent part, the following fused or otherwise connected domains or regions: a binding region construct, such as a binding domain or polypeptide, a connecting region construct including, for example, a native or engineered immunoglobulin hinge region polypeptide, and a tail region construct, including, for example, a construct that may comprise, consist essentially of, or consist of, a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide. According to certain embodiments that are particularly useful for gene therapy, the constructs, including fusion proteins, of the present invention may further comprise a native or engineered plasma membrane anchor domain. According to certain other preferred embodiments the constructs, including fusion proteins, of the present invention may further include a tail region having a native or engineered immunoglobulin heavy chain CH4 constant region polypeptide. In particularly preferred embodiments, the binding regions, such as polypeptide domains, of which the constructs, including binding domain-immunoglobulin fusion proteins, are comprised are, or are derived from, polypeptides that are the products of human gene sequences, but the invention need not be so limited and may in fact relate to constructs, including binding domain-immunoglobulin fusion proteins, as provided herein that are derived from any natural or artificial source, including genetically engineered and/or mutated polypeptides.

The present invention relates in part to the surprising observation that the novel constructs, including binding domain-immunoglobulin fusion proteins, described herein are capable of immunological activity. More specifically, these proteins retain the ability to participate in well known immunological effector activities including, for example, antibody dependent cell mediated cytotoxicity (*e.g.*, subsequent to antigen binding on a cell surface, engagement and induction of cytotoxic effector cells bearing appropriate Fc receptors, such as Natural Killer cells bearing FcR $\gamma$ III, under appropriate conditions) and/or complement fixation in complement dependent cytotoxicity (*e.g.*, subsequent to antigen binding on a cell surface, recruitment and activation of cytolytic proteins that are components of the blood complement cascade) despite having structures not be expected to be capable of promoting such effector activities or to promotion of such activities as



described herein. For reviews of ADCC and CDC see, *e.g.*, Carter, 2001 *Nat. Rev. Canc.* 1:118; Sulica *et al.*, 2001 *Int. Rev. Immunol.* 20:371; Maloney *et al.*, 2002 *Semin. Oncol.* 29:2; Sondel *et al.*, 2001 *Hematol Oncol Clin North Am* 15(4):703-21; Maloney 2001 *Anticanc. Drugs* 12 Suppl.2:1-4. IgA activation of complement by the alternative pathway is described, for example, in Schneiderman *et al.*, 1990 *J. Immunol.* 145:233.. As described in greater detail below, ADCC, complement fixation, and CDC are unexpected functions for constructs, including fusion proteins, comprising for example immunoglobulin heavy chain regions and having the structures described herein, and in particular for immunoglobulin fusion proteins comprising, for example, immunoglobulin hinge region polypeptides that are compromised in their ability to form interchain, homodimeric disulfide bonds.

Another advantage afforded by the present invention is constructs, including binding domain-immunoglobulin fusion polypeptides, of the invention that can be produced in substantial quantities that are typically greater than those routinely attained with single-chain antibody constructs of the prior art, for example. In preferred embodiments, constructs, including the binding domain-immunoglobulin fusion polypeptides, of the present invention are recombinantly expressed in mammalian or other desired and useful expression systems, which offer the advantage of providing polypeptides that are stable *in vivo* (*e.g.*, under physiological conditions). According to non-limiting theory, such stability may derive in part from posttranslational modifications, and specifically glycosylation. Production of the constructs, including binding domain-immunoglobulin fusion protein constructs, of the invention via recombinant mammalian expression has been attained in static cell cultures at a level of greater than 50 mg protein per liter culture supernatant and has been routinely observed in such cultures at 10-50 mg/liter, such that preferably at least 10-50 mg/liter may be produced under static culture conditions; also contemplated are enhanced production, in whole or in part, of the protein constructs of the invention using art-accepted scale-up methodologies such as “fed batch” (*i.e.*, non-static) production, where yields of at least 5-500 mg/l, and in some instances at least 0.5-1 gm/l, depending on the particular protein product, are obtained.

A construct, including a binding domain polypeptide, according to the present invention may be, for example, any polypeptide that possesses the ability to

specifically recognize and bind to a cognate biological molecule or complex of more than one molecule or assembly or aggregate, whether stable or transient, of such a molecule. Such molecules include proteins, polypeptides, peptides, amino acids, or derivatives thereof; lipids, fatty acids or the like, or derivatives thereof; carbohydrates, saccharides or the like or derivatives thereof; nucleic acids, nucleotides, nucleosides, purines, pyrimidines or related molecules, or derivatives thereof, or the like; or any combination thereof such as, for example, glycoproteins, glycopeptides, glycolipids, lipoproteins, proteolipids; or any other biological molecule that may be present in a biological sample. Biological samples may be provided, for example, by obtaining a blood sample, biopsy specimen, tissue explant, organ culture, biological fluid or any other tissue or cell or other preparation from a subject or a biological source. The subject or biological source may, for example, be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiable cell lines, transformed cell lines and the like, *etc.* In certain preferred embodiments of the invention, the subject or biological source may be suspected of having or being at risk for having a disease, disorder or condition, including a malignant disease, disorder or condition or a B cell disorder, which in certain further embodiments may be an autoimmune disease, and in certain other embodiments of the invention the subject or biological source may be known to be free of a risk or presence of such disease, disorder or condition.

A binding region, including a binding domain polypeptide, for example, may be any naturally occurring, synthetic, semi-synthetic, and/or recombinantly produced binding partner for a biological or other molecule that is a target structure of interest, herein sometimes referred to as an "antigen" but intended according to the present disclosure to encompass any target biological or other molecule to which it is desirable to have the subject invention, for example, a fusion protein, bind or specifically bind. Constructs of the invention, including binding domain-immunoglobulin fusion proteins, are defined to be "immunospecific" or capable of binding to a desired degree, including specifically binding, if they bind a desired target molecule such as an antigen as provided herein, at a desired level, for example, with a  $K_a$  of greater than or equal to about  $10^4 \text{ M}^{-1}$ , preferably of greater than or equal to about  $10^5 \text{ M}^{-1}$ , more preferably of greater than or equal to about  $10^6 \text{ M}^{-1}$  and still more preferably of greater than or equal to about  $10^7 \text{ M}^{-1}$ . Affinities of even greater than about  $10^7 \text{ M}^{-1}$  are still more preferred, such as affinities equal to or greater than

about about  $10^7$  M<sup>-1</sup>, about  $10^8$  M<sup>-1</sup>, and about  $10^9$  M<sup>-1</sup>, and about  $10^{10}$  M<sup>-1</sup>. Affinities of binding domain-immunoglobulin fusion proteins according to the present invention can be readily determined using conventional techniques, for example those described by Scatchard *et al.*, 1949 *Ann. N.Y. Acad. Sci.* **51**:660. Such determination of fusion protein  
5 binding to target antigens of interest can also be performed using any of a number of known methods for identifying and obtaining proteins that specifically interact with other proteins or polypeptides, for example, a yeast two-hybrid screening system such as that described in U.S. Patent No. 5,283,173 and U.S. Patent No. 5,468,614, or the equivalent.

Preferred embodiments of the subject invention constructs, for example,  
10 binding domain-immunoglobulin fusion proteins, comprise binding regions or binding domains that may include, for example, at least one native or engineered immunoglobulin variable region polypeptide, such as all or a portion or fragment of a native or engineered heavy chain and/or a native or engineered light chain V-region, provided it is capable of binding or specifically binding an antigen or other desired target structure of interest at a  
15 desired level of binding and selectivity. In other preferred embodiments the binding region or binding domain comprises, consists essentially of, or consists of, a single chain immunoglobulin-derived Fv product, for example, and scFv, which may include all or a portion of at least one native or engineered immunoglobulin light chain V-region and all or a portion of at least one native or engineered immunoglobulin heavy chain V-region, and a  
20 linker fused or otherwise connected to the V-regions; preparation and testing such constructs are described in greater detail herein. Other preparation and testing methods are well known in the art.

As described herein and known in the art, immunoglobulins comprise products of a gene family the members of which exhibit a high degree of sequence  
25 conservation. Amino acid sequences of two or more immunoglobulins or immunoglobulin domains or regions or portions thereof (*e.g.*, V<sub>H</sub> domains, V<sub>L</sub> domains, hinge regions, CH2 constant regions, CH3 constant regions) may be aligned and analyzed. Portions of sequences that correspond to one another may be identified, for instance, by sequence homology. Determination of sequence homology may be determined with any of a number  
30 of sequence alignment and analysis tools, including computer algorithms well known to those of ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, 1991 *J. Mol. Biol.* **219**:555-565; Henikoff and Henikoff, 1992 *Proc. Natl. Acad. Sci. USA* **89**:10915-

10919), which is available at the NCBI website (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST>). Default parameters may be used.

Portions, for example, of a particular immunoglobulin reference sequence and of any one or more additional immunoglobulin sequences of interest that may be compared to a reference sequence. “Corresponding” sequences, regions, fragments or the like, may be identified based on the convention for numbering immunoglobulin amino acid positions according to Kabat, *Sequences of Proteins of Immunological Interest*, (5<sup>th</sup> ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)). For example, according to this convention, the immunoglobulin family to which an immunoglobulin sequence of interest belongs is determined based on conservation of variable region polypeptide sequence invariant amino acid residues, to identify a particular numbering system for the immunoglobulin family, and the sequence(s) of interest can then be aligned to assign sequence position numbers to the individual amino acids which comprise such sequence(s). Preferably at least about 70%, more preferably at least about 80%-85% or about 86%-89%, and still more preferably at least about 90%, about 92%, about 94%, about 96%, about 98% or about 99% of the amino acids in a given amino acid sequence of at least about 1000, more preferably about 700-950, more preferably about 350-700, still more preferably about 100-350, still more preferably about 80-100, about 70-80, about 60-70, about 50-60, about 40-50 or about 30-40 consecutive amino acids of a sequence, are identical to the amino acids located at corresponding positions in a reference sequence such as those disclosed by Kabat (1991) or in a similar compendium of related immunoglobulin sequences, such as may be generated from public databases (*e.g.*, Genbank, SwissProt, *etc.*) using sequence alignment tools such as, for example, those described above. In certain preferred embodiments, an immunoglobulin sequence of interest or a region, portion, derivative or fragment thereof is greater than about 95% identical to a corresponding reference sequence, and in certain preferred embodiments such a sequence of interest may differ from a corresponding reference at no more than about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid positions.

For example, in certain embodiments the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in

pertinent part a human or other species immunoglobulin heavy chain variable region polypeptide comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in, for example, SEQ ID NO:\_\_, which comprises, for example, a murine V<sub>H</sub>-  
5 derived sequence. At a relatively limited number of immunoglobulin V<sub>H</sub> sequence positions, for example, including position 11, amino acid conservation is observed in the overwhelming majority of V<sub>H</sub> sequences analyzed across mammalian species lines (e.g., Leu11, Val37, Gly44, Leu45, Trp47; Nguyen *et al.*, 1998 *J. Mol. Biol.* **275**:413). Various such amino acid residues, and hence their side chains, are located at the surface of the  
10 variable domain (V<sub>H</sub>). They may contact residues of the C<sub>H</sub>1 (e.g., Leu11) and the V<sub>L</sub> domains (e.g., Val37, Gly44, Leu45, and Trp47) and may, in the absence of light chains, contribute to stability and solubility of the protein (see, e.g., Chothia *et al.*, 1985 *J. Mol. Biol.* **186**:651; Muyldermans *et al.*, 1994 *Prot. Engineer.* **7**:1129; Desmyter *et al.*, 1996 *Nat. Struct. Biol.* **3**:803; Davies *et al.*, 1994 *FEBS Lett.* **339**:285). In certain embodiments, for  
15 example, the present invention is also directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 12, 80,  
20 81, 82, 83, 105, 106, 107 and 108. In still other certain embodiments, for example, the present invention is directed to a construct, including a binding domain-immunoglobulin fusion protein, comprising in pertinent part (1) a human immunoglobulin heavy chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said heavy  
25 chain sequence having a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, and (2) a human immunoglobulin light chain variable region polypeptide, or an immunoglobulin light chain variable region polypeptide from another species, comprising, consisting essentially of, or consisting of, said light chain sequence having a mutation,

alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108.

As another example, by reference to immunoglobulin sequence compendia and databases such as those cited above, for example, the relatedness of two or more immunoglobulin sequences to each other can readily and without undue experimentation be established in a manner that permits identification of the animal species of origin, the class and subclass (*e.g.*, isotype) of a particular immunoglobulin or immunoglobulin region polypeptide sequence. Any immunoglobulin variable region polypeptide sequence, including native or engineered V<sub>H</sub> and/or V<sub>L</sub> and/or single-chain variable region (sFv) sequences or other native or engineered V region-derived sequences or the like, may be used as a binding region or binding domain. Engineered sequences includes immunoglobulin sequences from any species, preferably human or mouse, for example, that include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112 in a heavy chain variable region sequence or an scFv, and/or a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence or an scFv.

Various preferred embodiments include, for example, native or engineered immunoglobulin V region polypeptide sequences derived, for example, from antibodies including monoclonal antibodies such as murine or other rodent antibodies, or antibodies or monoclonal antibodies derived from other sources such as goat, rabbit, equine, bovine, camelid or other species, including transgenic animals, and also including human or humanized antibodies or monoclonal antibodies. Non-limiting examples include variable region polypeptide sequences derived from monoclonal antibodies such as those referenced herein and/or described in greater detail in the Examples below, for instance, CD20-binding or specific murine monoclonal antibodies (*e.g.*, 2H7), monoclonal antibody L6 (specific for a carbohydrate-defined epitope and available from American Type Culture Collection, Manassas, VA, as hybridoma HB8677), and monoclonal antibodies that bind to or are specific for CD28 (*e.g.*, monoclonal antibody 2E12), CD40, CD80, CD137 (*e.g.*,

monoclonal antibody 5B9 or monoclonal antibody 1D8 which recognizes the murine homologue of CD137, 41BB) and CD152 (CTLA-4).

Other binding regions, including binding domain polypeptides, may comprise any protein or portion thereof that retains the ability to bind or specifically bind to an antigen as provided herein, including non-immunoglobulins. Accordingly the invention contemplates constructs, including fusion proteins, comprising binding region or binding domain polypeptides that are derived from polypeptide ligands such as hormones, cytokines, chemokines, and the like; cell surface or soluble receptors for such polypeptide ligands; lectins; intercellular adhesion receptors such as specific leukocyte integrins, selectins, immunoglobulin gene superfamily members, intercellular adhesion molecules (ICAM-1, -2, -3) and the like; histocompatibility antigens; *etc.*

Examples of cell surface receptors useful in the preparation of, or as, binding regions, or that may provide a binding domain polypeptide, and that may also be selected as a target molecule or antigen to which a construct, including for example, a binding domain-Ig fusion protein of the present invention desirably binds, include the following, or the like: HER1 (*e.g.*, GenBank Accession Nos. U48722, SEG\_HEGFREXS, KO3193), HER2 (Yoshino *et al.*, 1994 *J. Immunol.* 152:2393; Disis *et al.*, 1994 *Canc. Res.* 54:16; see also, *e.g.*, GenBank Acc. Nos. X03363, M17730, SEG\_HUMHER20), HER3 (*e.g.*, GenBank Acc. Nos. U29339, M34309), HER4 (Plowman *et al.*, 1993 *Nature* 366:473; see also *e.g.*, GenBank Acc. Nos. L07868, T64105), epidermal growth factor receptor (EGFR) (*e.g.*, GenBank Acc. Nos. U48722, SEG\_HEGFREXS, KO3193), vascular endothelial cell growth factor (*e.g.*, GenBank No. M32977), vascular endothelial cell growth factor receptor (*e.g.*, GenBank Acc. Nos. AF022375, 1680143, U48801, X62568), insulin-like growth factor-I (*e.g.*, GenBank Acc. Nos. X00173, X56774, X56773, X06043, *see also* European Patent No. GB 2241703), insulin-like growth factor-II (*e.g.*, GenBank Acc. Nos. X03562, X00910, SEG\_HUMGFIA, SEG\_HUMGFI2, M17863, M17862), transferrin receptor (Trowbridge and Omary, 1981 *Proc. Nat. Acad. USA* 78:3039; see also *e.g.*, GenBank Acc. Nos. X01060, M11507), estrogen receptor (*e.g.*, GenBank Acc. Nos. M38651, X03635, X99101, U47678, M12674), progesterone receptor (*e.g.*, GenBank Acc. Nos. X51730, X69068, M15716), follicle stimulating hormone receptor (FSH-R) (*e.g.*, GenBank Acc. Nos. Z34260, M65085),

retinoic acid receptor (e.g., GenBank Acc. Nos. L12060, M60909, X77664, X57280, X07282, X06538), MUC-1 (Barnes *et al.*, 1989 *Proc. Nat. Acad. Sci. USA* **86**:7159; see also e.g., GenBank Acc. Nos. SEG\_MUSMUCIO, M65132, M64928) NY-ESO-1 (e.g., GenBank Acc. Nos. AJ003149, U87459), NA 17-A (e.g., European Patent No. WO 96/40039), Melan-A/MART-1 (Kawakami *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* **91**:3515; see also e.g., GenBank Acc. Nos. U06654, U06452), tyrosinase (Topalian *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* **91**:9461; see also e.g., GenBank Acc. Nos. M26729, SEG\_HUMTYR0, see also Weber *et al.*, *J. Clin. Invest* (1998) **102**:1258), Gp-100 (Kawakami *et al.*, 1994 *Proc. Nat. Acad. Sci. USA* **91**:3515; see also e.g., GenBank Acc. No. S73003, see also European Patent No. EP 668350; Adema *et al.*, 1994 *J. Biol. Chem.* **269**:20126), MAGE (van den Bruggen *et al.*, 1991 *Science* **254**:1643; see also e.g., GenBank Acc. Nos. U93163, AF064589, U66083, D32077, D32076, D32075, U10694, U10693, U10691, U10690, U10689, U10688, U10687, U10686, U10685, L18877, U10340, U10339, L18920, U03735, M77481), BAGE (e.g., GenBank Acc. No. U19180; see also U.S. Patent Nos. 5,683,886 and 5,571,711), GAGE (e.g., GenBank Acc. Nos. AF055475, AF055474, AF055473, U19147, U19146, U19145, U19144, U19143, U19142), any of the CTA class of receptors including in particular HOM-MEL-40 antigen encoded by the SSX2 gene (e.g., GenBank Acc. Nos. X86175, U90842, U90841, X86174), carcinoembryonic antigen (CEA, Gold and Freedman, 1985 *J. Exp. Med.* **121**:439; see also e.g., GenBank Acc. Nos. SEG\_HUMCEA, M59710, M59255, M29540), and PyLT (e.g., GenBank Acc. Nos. J02289, J02038).

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may be targets, including target antigens, include the following, or the like: CD2 (e.g., GenBank Acc. Nos. Y00023, SEG\_HUMCD2, M16336, M16445, SEG\_MUSCD2, M14362), 4-1BB (CDw137, Kwon *et al.*, 1989 *Proc. Nat. Acad. Sci. USA* **86**:1963, 4-1BB ligand (Goodwin *et al.*, 1993 *Eur. J. Immunol.* **23**:2361; Melero *et al.*, 1998 *Eur. J. Immunol.* **3**:116), CD5 (e.g., GenBank Acc. Nos. X78985, X89405), CD10 (e.g., GenBank Acc. Nos. M81591, X76732) CD27 (e.g., GenBank Acc. Nos. M63928, L24495, L08096), CD28 (June *et al.*, 1990 *Immunol. Today* **11**:211; see also, e.g., GenBank Acc. Nos. J02988, SEG\_HUMCD28, M34563), CD152/CTLA-4 (e.g., GenBank Acc. Nos. L15006, X05719, SEG\_HUMIGCTL), CD40



(e.g., GenBank Acc. Nos. M83312, SEG\_MUSC040A0, Y10507, X67878, X96710, U15637, L07414), interferon- $\gamma$  (IFN- $\gamma$ ; see, e.g., Farrar *et al.* 1993 *Ann. Rev. Immunol.* **11**:571 and references cited therein, Gray *et al.* 1982 *Nature* **295**:503, Rinderknecht *et al.* 1984 *J. Biol. Chem.* **259**:6790, DeGrado *et al.* 1982 *Nature* **300**:379), interleukin-4 (IL-4; see, e.g., 53<sup>rd</sup> Forum in Immunology, 1993 *Research in Immunol.* **144**:553-643; Banchereau *et al.*, 1994 in *The Cytokine Handbook*, 2<sup>nd</sup> ed., A. Thomson, ed., Academic Press, NY, p. 99; Keegan *et al.*, 1994 *J. Leukocyt. Biol.* **55**:272, and references cited therein), interleukin-17 (IL-17) (e.g., GenBank Acc. Nos. U32659, U43088) and interleukin-17 receptor (IL-17R) (e.g., GenBank Acc. Nos. U31993, U58917). Notwithstanding the foregoing, the present invention expressly does not encompass any immunoglobulin fusion protein that is disclosed in U.S. 5,807,734, or U.S. 5,795,572.

Additional cell surface receptors that may be sources of binding region or binding domain polypeptides or portions thereof, or that may serve as targets including target antigens or binding sites include the following, or the like: CD59 (e.g., GenBank Acc. Nos. SEG\_HUMCD590, M95708, M34671), CD48 (e.g., GenBank Acc. Nos. M59904), CD58/LFA-3 (e.g., GenBank Acc. No. A25933, Y00636, E12817; see also JP 1997075090-A), CD72 (e.g., GenBank Acc. Nos. AA311036, S40777, L35772), CD70 (e.g., GenBank Acc. Nos. Y13636, S69339), CD80/B7.1 (Freeman *et al.*, 1989 *J. Immunol.* **43**:2714; Freeman *et al.*, 1991 *J. Exp. Med.* **174**:625; see also e.g., GenBank Acc. Nos. U33208, I683379), CD86/B7.2 (Freeman *et al.*, 1993 *J. Exp. Med.* **178**:2185, Boriello *et al.*, 1995 *J. Immunol.* **155**:5490; see also, e.g., GenBank Acc. Nos. AF099105, SEG\_MMB72G, U39466, U04343, SEG\_HSB725, L25606, L25259), B7-H1/B7-DC (e.g., Genbank Acc. Nos. NM\_014143, AF177937, AF317088; Dong *et al.*, 2002 *Nat. Med.* Jun 24 [epub ahead of print], PMID 12091876; Tseng *et al.*, 2001 *J. Exp. Med.* **193**:839; Tamura *et al.*, 2001 *Blood* **97**:1809; Dong *et al.*, 1999 *Nat. Med.* **5**:1365), CD40 ligand (e.g., GenBank Acc. Nos. SEG\_HUMCD40L, X67878, X65453, L07414), IL-17 (e.g., GenBank Acc. Nos. U32659, U43088), CD43 (e.g., GenBank Acc. Nos. X52075, J04536), ICOS (e.g., Genbank Acc. No. AH011568), CD3 (e.g., Genbank Acc. Nos. NM\_000073 (gamma subunit), NM\_000733 (epsilon subunit), X73617 (delta subunit)), CD4 (e.g., Genbank Acc. No. NM\_000616), CD25 (e.g., Genbank Acc. No. NM\_000417), CD8 (e.g., Genbank Acc. No. M12828),

CD11b (*e.g.*, Genbank Acc. No. J03925), CD14 (*e.g.*, Genbank Acc. No. XM\_039364), CD56 (*e.g.*, Genbank Acc. No. U63041), CD69 (*e.g.*, Genbank Acc. No. NM\_001781) and VLA-4 ( $\alpha_4\beta_7$ ) (*e.g.*, GenBank Acc. Nos. L12002, X16983, L20788, U97031, L24913, M68892, M95632). The following cell surface receptors are typically associated with B cells: CD19 (*e.g.*, GenBank Acc. Nos. SEG\_HUMCD19W0, M84371, SEG\_MUSCD19W, M62542), CD20 (*e.g.*, GenBank Acc. Nos. SEG\_HUMCD20, M62541), CD22 (*e.g.*, GenBank Acc. Nos. I680629, Y10210, X59350, U62631, X52782, L16928), CD30 (*e.g.*, Genbank Acc. Nos. M83554, D86042), CD153 (CD30 ligand, *e.g.*, GenBank Acc. Nos. L09753, M83554), CD37 (*e.g.*, GenBank Acc. Nos. SEG\_MMCD37X, X14046, X53517), CD50 (ICAM-3, *e.g.*, GenBank Acc. No. NM\_002162), CD106 (VCAM-1) (*e.g.*, GenBank Acc. Nos. X53051, X67783, SEG\_MMVCAM1C, *see also* U.S. Patent No. 5,596,090), CD54 (ICAM-1) (*e.g.*, GenBank Acc. Nos. X84737, S82847, X06990, J03132, SEG\_MUSICAM0), interleukin-12 (*see, e.g.*, Reiter *et al*, 1993 *Crit. Rev. Immunol.* 13:1, and references cited therein), CD134 (OX40, *e.g.*, GenBank Acc. No. AJ277151), CD137 (41BB, *e.g.*, GenBank Acc. No. L12964, NM\_001561), CD83 (*e.g.*, GenBank Acc. Nos. AF001036, AL021918), DEC-205 (*e.g.*, GenBank Acc. Nos. AF011333, U19271).

Constructs, including binding domain-immunoglobulin fusion proteins, of the present invention comprise, for example, a binding domain, such as a binding domain polypeptide that, according to certain particularly preferred embodiments, is capable of binding or specifically binding at least one target, for example, a target antigen or other binding site that is present on an immune effector cell. According to non-limiting theory, such constructs, including for example binding domain- immunoglobulin fusion proteins, may advantageously recruit desired immune effector cell function(s) in a therapeutic context, where it is well known that immune effector cells having different specialized immune functions can be identified or distinguished from one another on the basis of their differential expression of a wide variety of cell surface antigens, including many of the antigens described herein to which constructs of the invention including binding domain polypeptides can specifically bind. As noted herein, immune effector cells include any cell that is capable of directly mediating an activity which is a component of immune system

function, including cells having such capability naturally or as a result of genetic engineering.

In certain embodiments an immune effector cell comprises a cell surface receptor for an immunoglobulin or other peptide binding molecule, such as a receptor for an immunoglobulin constant region and including the class of receptors commonly referred to as "Fc receptors" ("FcR"s). A number of FcRs have been structurally and/or functionally characterized and are well known in the art, including FcR having specific abilities to interact with a restricted subset of immunoglobulin heavy chain isotypes, or that interact with Fc domains with varying affinities, and/or which may be expressed on restricted subsets of immune effector cells under certain conditions (*e.g.*, Kijimoto-Ochichai *et al.*, 2002 *Cell Mol. Life Sci.* **59**:648; Davis *et al.*, 2002 *Curr. Top. Microbiol. Immunol.* **266**:85; Pawankar, 2001 *Curr. Opin. Allerg. Clin. Immunol.* **1**:3; Radaev *et al.*, 2002 *Mol. Immunol.* **38**:1073; Wurzburg *et al.*, 2002 *Mol. Immunol.* **38**:1063; Sulica *et al.*, 2001 *Int. Rev. Immunol.* **20**:371; Underhill *et al.*, 2002 *Ann. Rev. Immunol.* **20**:825; Coggeshall, 2002 *Curr. Dir. Autoimm.* **5**:1; Mimura *et al.*, 2001 *Adv. Exp. Med. Biol.* **495**:49; Baumann *et al.*, 2001 *Adv. Exp. Med. Biol.* **495**:219; Santoso *et al.*, 2001 *Ital. Heart J.* **2**:811; Novak *et al.*, 2001 *Curr. Opin. Immunol.* **13**:721; Fossati *et al.*, 2001 *Eur. J. Clin. Invest.* **31**:821).

Cells that are capable of mediating ADCC are preferred examples of immune effector cells according to the present invention. Other preferred examples include Natural Killer cells, tumor-infiltrating T lymphocytes (TILs), cytotoxic T lymphocytes, and granulocytic cells such as cells that comprise allergic response mechanisms. Immune effector cells thus include, but are not limited to, cells of hematopoietic origins including cells at various stages of differentiation within myeloid and lymphoid lineages and which may (but need not) express one or more types of functional cell surface FcR, such as T lymphocytes, B lymphocytes, NK cells, monocytes, macrophages, dendritic cells, neutrophils, basophils, eosinophils, mast cells, platelets, erythrocytes, and precursors, progenitors (*e.g.*, hematopoietic stem cells), as well as quiescent, activated, and mature forms of such cells. Other immune effector cells may include cells of non-hematopoietic origin that are capable of mediating immune functions, for example, endothelial cells, keratinocytes, fibroblasts, osteoclasts, epithelial cells, and other cells. Immune effector cells

may also include cells that mediate cytotoxic or cytostatic events, or endocytic, phagocytic, or pinocytotic events, or that effect induction of apoptosis, or that effect microbial immunity or neutralization of microbial infection, or cells that mediate allergic, inflammatory, hypersensitivity and/or autoimmune reactions.

5 Allergic response mechanisms are well known in the art and include an antigen (*e.g.*, allergen)-specific component such as an immunoglobulin (*e.g.*, IgE), as well as the cells and mediators which comprise sequelae to allergen-immunoglobulin (*e.g.*, IgE) encounters (*e.g.*, Ott *et al.*, 2000 *J. Allerg. Clin. Immunol.* **106**:429; Barnes, 2000 *J. Allerg. Clin. Immunol.* **106**:5; Togias, 2000 *J. Allerg. Clin. Immunol.* **105**:S599; Akdis *et al.*, 2000  
10 *Int. Arch. Allerg. Immunol.* **121**:261; Beach, 2000 *Occup. Med.* **15**:455). Particularly with regard to constructs, including binding domain-immunoglobulin fusion proteins, of the present invention that interact with FcR, certain embodiments of the present invention contemplate constructs including fusion proteins that comprise one or more IgE-derived domains including, for example, those that are capable of inducing an allergic response  
15 mechanism that comprises IgE-specific FcR, or portions thereof, which IgE-specific FcRs include those noted above and described or identified in the cited articles. Without wishing to be bound by particular theory or mechanism, and as disclosed herein, constructs, including fusion proteins, of the present invention may comprise portions of IgE heavy chain Fc domain polypeptides, for example, native or engineered IgE CH3 and CH4  
20 domains, whether provided or expressed as cell surface proteins (*e.g.*, with a plasma membrane anchor domain) or as soluble or otherwise not cell-bound proteins (*e.g.*, without a plasma membrane anchor domain). Further according to non-limiting theory, recruitment and induction of an allergic response mechanism (*e.g.*, an FcR-epsilon expressing immune effector cell) may proceed as the result of either or both of the presence of an IgE Fc domain  
25 or portion thereof as described herein (*e.g.*, one that is capable of triggering an allergic mechanism by FcR crosslinking) and the presence of a target such as a antigen to which the binding region, for example a binding domain, binds or specifically binds. The present invention therefore exploits induction of allergic response mechanisms in heretofore unappreciated contexts, such as treatment of a malignant condition or a B cell disorder,  
30 including those described or referenced herein.

An immunoglobulin hinge region polypeptide includes any hinge peptide or polypeptide that occurs naturally, as an artificial peptide or as the result of genetic engineering and that is situated, for example, in an immunoglobulin heavy chain polypeptide between the amino acid residues responsible for forming intrachain immunoglobulin-domain disulfide bonds in CH1 and CH2 regions. Hinge region polypeptides for use in the present invention may also include a mutated or otherwise altered hinge region polypeptide. Accordingly, for example, an immunoglobulin hinge region polypeptide may be derived from, or may be a portion or fragment of (*i.e.*, one or more amino acids in peptide linkage, typically about 15-115 amino acids, preferably about 95-110, about 80-94, about 60-80, or about 5-65 amino acids, preferably about 10-50, more preferably about 15-35, still more preferably about 18-32, still more preferably about 20-30, still more preferably about 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acids) an immunoglobulin polypeptide chain region classically regarded as having hinge function, including those described herein, but a hinge region polypeptide for use in the instant invention need not be so restricted and may include one or more amino acids situated (according to structural criteria for assigning a particular residue to a particular domain that may vary, as known in the art) in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH2 domain in the cases of IgG, IgA and IgD (or in an adjoining immunoglobulin domain such as a CH1 domain and/or a CH3 domain in the case of IgE), or in the case of certain artificially engineered immunoglobulin constructs, an immunoglobulin variable region domain.

Wild-type immunoglobulin hinge region polypeptides include any known or later-discovered naturally occurring hinge region that is located between the constant region domains, CH1 and CH2, of an immunoglobulin, for example, a human immunoglobulin (or between the CH1 and CH3 regions of certain types of immunoglobulins, such as IgE),. For use in constructing one type of connecting region, the wild-type immunoglobulin hinge region polypeptide is preferably a human immunoglobulin hinge region polypeptide, preferably comprising a hinge region from a human IgG, IgA, or IgD immunoglobulin (or the CH2 region of an IgE immunoglobulin), and more preferably, for example, a hinge region polypeptide from a wild-type or mutated human IgG1 isotype as described herein.

As is known to the art, despite the tremendous overall diversity in immunoglobulin amino acid sequences, immunoglobulin primary structure exhibits a high degree of sequence conservation in particular portions of immunoglobulin polypeptide chains, notably with regard to the occurrence of cysteine residues which, by virtue of their  
5 sulfhydryl groups, offer the potential for disulfide bond formation with other available sulfhydryl groups. Accordingly, in the context of the present invention wild-type immunoglobulin hinge region polypeptides for use as connecting regions include those that feature one or more highly conserved (*e.g.*, prevalent in a population in a statistically significant manner) cysteine residues, and in certain preferred embodiments a connecting  
10 region may comprise, or consist essentially of, or consist of, a mutated hinge region polypeptide may be selected that contains less than the number of naturally-occurring cysteines, for example, zero or one or two cysteine residue(s) in the case of IgG1 and IgG4 hinge regions, and that is derived or constructed from (or using) such a wild-type hinge region sequence.

15 In certain preferred embodiments wherein the connecting region is a hinge region polypeptide and the hinge region polypeptide is a mutated, engineered or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is derived or constructed from (or using) a wild-type hinge region sequence, it is noted that the wild-type human IgG1 hinge region polypeptide sequence comprises three non-adjacent cysteine residues, referred  
20 to as a first cysteine of the wild-type hinge region, a second cysteine of the wild-type hinge region and a third cysteine of the wild-type hinge region, respectively, proceeding along the hinge region sequence from the polypeptide N-terminus toward the C-terminus. This can be referred to herein as a "CCC" hinge (or a "WTH", *i.e.*, a wild-type hinge). Examples of mutated or engineered hinge regions include those with no cysteines, which may be referred  
25 to herein as an "XXX" hinge (or, for example, as "MH-XXX," referring to a mutant or engineered hinge with three amino acids or other molecules in place of naturally occurring cysteines, such as, for example, "MH-SSS", which refers to a mutant hinge with three serine residues in place of the naturally occurring cysteine residues. It will be understood that the term "mutant" refers only to the fact that a different molecule or molecules is present, or no  
30 molecule, at the position of a naturally occurring residue and does not refer to any particular

method by which such substitution, alteration, or deletion has been carried out. Accordingly, in certain embodiments of the present invention, the connecting region may be a hinge region polypeptide and the hinge region polypeptide is a mutated human IgG1 immunoglobulin hinge region polypeptide that contains two cysteine residues and in which the first cysteine of the wild-type hinge region has not changed or deleted, for example. This can be referred to as a "MH-CXX" hinge, for example, a "MH-CSC" hinge, in which case the cysteine residue has been replaced with a serine residue. In certain other embodiments of the present invention the mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue and include, for example, a "MH-CSS" hinge or a "MH-SSC" hinge or a "MH-CSC" hinge, and in certain other embodiments the mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues such as, for example, a "MH-SSS" hinge.

The constructs, including binding domain-immunoglobulin fusion proteins, of the present invention expressly do not contemplate any fusion protein that is disclosed in U.S. Patent No. 5,892,019. U.S. Patent No. 5,892,019 refers to a human IgG1 hinge region in which the first IgG1 hinge region cysteine residue has been changed or deleted, but retains both of the second and third IgG1 hinge region cysteine residues that correspond to the second and third cysteines of the wild-type IgG1 hinge region sequence. The patent states that the first cysteine residue of the wild-type IgG1 hinge region is replaced to prevent interference by the first cysteine residue with proper assembly of the polypeptide described therein into a dimer. The patent requires that the second and third cysteines of the IgG1 hinge region be retained to provide interchain disulfide linkage between two heavy chain constant regions to promote dimer formation so that the molecule contains has effector function such as the ability to mediate ADCC.

By contrast and as described herein, the constructs, including the binding domain-immunoglobulin fusion proteins, of the present invention, various of which are capable of ADCC, CDC and/or complement fixation, for example, are not so limited and may comprise, in pertinent part, for example, (i) a wild-type immunoglobulin hinge region polypeptide, such as a wild-type human immunoglobulin hinge region polypeptide, for example, a human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated or

otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated or deleted, (iii) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that, for example, is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, or (iv) a mutated or otherwise altered immunoglobulin hinge region polypeptide, such as a mutated or otherwise altered human immunoglobulin hinge region polypeptide, for example, a mutated or otherwise altered human IgG1 immunoglobulin hinge region polypeptide that is or has been derived or constructed from (or using) a wild-type immunoglobulin hinge region polypeptide or nucleic acid sequence having three or more cysteine residues, wherein the mutated or otherwise altered (for example, by amino acid change or deletion) human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues. The present invention offers unexpected advantages associated with retention by the constructs, including the fusion proteins, described herein of the ability to mediate ADCC and/or CDC and/or complement fixation notwithstanding that the ability to dimerize via IgG1 hinge region interchain disulfide bonds is ablated or compromised by the removal or replacement of one, two or three hinge region cysteine residues, and even in constructs where the first cysteine of an IgG1 hinge region, for example, is not mutated or otherwise altered or deleted.

A connecting region may comprise a mutated or otherwise altered immunoglobulin hinge region polypeptide, which itself may comprise a hinge region that



has its origin in an immunoglobulin of a species, of an immunoglobulin isotype or class, or of an immunoglobulin subclass that is different from that of the tail region, for example, a tail region comprising, or consisting essentially of, or consisting of, CH2 and CH3 domains (or IgE CH3 and CH4 domains). For instance, in certain embodiments of the invention, a construct, for example, a binding domain-immunoglobulin fusion protein, may comprise a binding region such as a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region polypeptide comprising, or consisting essentially of, or consisting of, a wild-type human IgA hinge region polypeptide, or a mutated or otherwise altered human IgA hinge region polypeptide that contains zero or only one or more cysteine residues (but less than the wild-type number of cysteines), as described herein, or a wild-type human IgG hinge, such as an IgG1 hinge, region polypeptide, or a wild-type human IgE hinge-acting region, *i.e.*, IgE CH2 region polypeptide, or a mutated or otherwise altered human IgG hinge, such as an IgG1 hinge, region polypeptide that is or has been mutated or otherwise altered to contain zero, one or two cysteine residues wherein the first cysteine of the wild-type hinge region is not mutated or altered or deleted, as also described herein. Such a hinge region polypeptide may be fused or otherwise connected to, for example, a tail region comprising, or consisting essentially of, or consisting of, an immunoglobulin heavy chain CH2 region polypeptide from a different Ig isotype or class, for example an IgA or an IgD or an IgG subclass (or a CH3 region from an IgE subclass), which in certain preferred embodiments will be the IgG1 or IgA or IgE subclass and in certain other preferred embodiments may be any one of the IgG2, IgG3 or IgG4 subclasses.

For example, and as described in greater detail herein, in certain embodiments of the present invention a connecting region may be selected to be an immunoglobulin hinge region polypeptide, which is or has been derived from a wild-type human IgA hinge region that naturally comprises three cysteines, where the selected hinge region polypeptide is truncated or otherwise altered or substituted relative to the complete and/or naturally-occurring hinge region such that only one or two of the cysteine residues remain (*e.g.*, SEQ ID NOS:35-36). Similarly, in certain other embodiments of the invention, the construct may be binding domain-immunoglobulin fusion protein comprising a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge region

polypeptide comprising a mutated or otherwise altered hinge region polypeptide in which the number of cysteine residues is reduced by amino acid substitution or deletion, for example a mutated or otherwise altered IgG1 hinge region containing zero, one or two cysteine residues as described herein, or an IgD hinge region containing zero cysteine residues.

A mutated or otherwise altered hinge region polypeptide may thus be derived or constructed from (or using) a wild-type immunoglobulin hinge region that contains one or more cysteine residues. In certain embodiments, a mutated or otherwise altered hinge region polypeptide may contain zero or only one cysteine residue, wherein the mutated or otherwise altered hinge region polypeptide is or has been derived from a wild type immunoglobulin hinge region that contains, respectively, one or more or two or more cysteine residues. In the mutated or otherwise altered hinge region polypeptide, the cysteine residues of the wild-type immunoglobulin hinge region are preferably deleted or substituted with amino acids that are incapable of forming a disulfide bond. In one embodiment of the invention, a mutated or otherwise altered hinge region polypeptide is or has been derived from a human IgG wild-type hinge region polypeptide, which may include any of the four human IgG isotype subclasses, IgG1, IgG2, IgG3 or IgG4. In certain preferred embodiments, the mutated or otherwise altered hinge region polypeptide is or has been derived from (or using) a human IgA or IgD wild-type hinge region polypeptide. By way of example, a mutated or otherwise altered hinge region polypeptide that is or has been derived from a human IgG1 or IgA wild-type hinge region polypeptide may comprise mutations, alterations, or deletions at two of the three cysteine residues in the wild-type immunoglobulin hinge region, or mutations, alterations, or deletions at all three cysteine residues.

The cysteine residues that are present in a wild-type immunoglobulin hinge region and that are removed or altered by mutagenesis or any other techniques according to particularly preferred embodiments of the present invention include cysteine residues that form, or that are capable of forming, interchain disulfide bonds. Without wishing to be bound by particular theory or mechanism of action, the present invention contemplates that mutation, deletion, or other alteration of such hinge region cysteine residues, which are

believed to be involved in formation of interchain disulfide bridges, reduces the ability of the subject invention binding domain-immunoglobulin fusion protein to dimerize (or form higher oligomers) via interchain disulfide bond formation, while surprisingly not ablating or undesireably compromising the ability of a fusion protein or other construct to promote ADCC, and/or CDC and/or to fix complement. In particular, the Fc receptors that mediate ADCC (*e.g.*, FcRIII, CD16) exhibit low affinity for immunoglobulin Fc domains, supporting the idea that functional binding of Fc to FcR requires avidity stabilization of the Fc-FcR complex by virtue of the dimeric structure of heavy chains in a conventional antibody, and/or FcR aggregation and cross-linking by a conventional antibody Fc structure.

Sonderman *et al.*, 2000 *Nature* **406**:267; Radaev *et al.*, 2001 *J. Biol. Chem.* **276**:16469; Radaev *et al.*, 2001 *J. Biol. Chem.* **276**:16478; Koolwijk *et al.*, 1989 *J. Immunol.* **143**:1656; Kato *et al.*, 2000 *Immunol. Today* **21**:310. Hence, the constructs, including for example binding domain-immunoglobulin fusion proteins, of the present invention provide the advantages associated with single-chain constructs including single-chain immunoglobulin fusion proteins while also unexpectedly retaining one or more immunological activities. Similarly, the ability to fix complement is typically associated with immunoglobulins that are dimeric with respect to heavy chain constant regions such as those that comprise Fc, while various constructs, including binding domain-immunoglobulin fusion proteins, of the present invention, which may, due to the replacement or deletion of hinge region cysteine residues or due to other structural modifications as described herein, for example, have compromised or ablated abilities to form interchain disulfide bonds, exhibit the unexpected ability to fix complement. Additionally, according to certain embodiments of the present invention wherein a construct, including, for example, a binding domain-immunoglobulin fusion protein, may comprise a connecting region and tail region comprising, or consisting essentially of, or consisting of, one or more of a human IgE hinge-acting region, *i.e.*, a IgE CH2 region polypeptide, a human IgE CH3 constant region polypeptide, and a human IgE CH4 constant region polypeptide, the invention constructs including fusion proteins unexpectedly retain the immunological activity of mediating ADCC and/or of inducing an allergic response mechanism.

Selection of an immunoglobulin hinge region polypeptide as a connecting region according to certain embodiments of the subject invention constructs, such as binding domain-immunoglobulin fusion proteins, may relate to the use of an “alternative hinge region” polypeptide sequence, which includes a polypeptide sequence that is not necessarily derived from any immunoglobulin hinge region sequence *per se*. Instead, an alternative hinge region refers to a hinge region polypeptide that comprises an amino acid sequence, or other molecular sequence, of at least about ten consecutive amino acids or molecules, and in certain embodiments at least about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30, 31-50, 51-60, 71-80, 81-90, or 91-110 amino acids or molecules that is present in a sequence selected from any one of SEQ ID NOS: \_\_-\_\_, for example a polypeptide sequence that is or has been derived from a region located between intrachain disulfide-generated immunoglobulin-like loop domains of immunoglobulin gene superfamily members such as CD2 (*e.g.*, Genbank Acc. No. NM\_001767), CD4 (*e.g.*, Genbank Acc. No. NM\_000616), CD5 (*e.g.*, Genbank Acc. No. BC027901), CD6 (*e.g.*, Genbank Acc. No. NM\_006725), CD7 (*e.g.*, Genbank Acc. Nos. XM\_046782, BC009293, NM\_006137) or CD8 (*e.g.*, Genbank Acc. No. M12828), or other Ig superfamily members. By way of non-limiting example, an alternative hinge region used as a connecting region, for example, may provide a glycosylation site as provided herein, or may provide a human gene-derived polypeptide sequence for purposes of enhancing the degree of “humanization” of a fusion protein, or may comprise, or consist essentially of, or consist of, an amino acid sequence that eliminates or reduces the ability of a construct of the invention, such as a fusion protein, to form multimers or oligomers or aggregates or the like. Certain alternative hinge region polypeptide sequences, including those described herein, may be derived or constructed from (or using) the polypeptide sequences of immunoglobulin gene superfamily members that are not actual immunoglobulins *per se*. For instance and according to non-limiting theory, certain polypeptide sequences that are situated between intrachain disulfide-generated immunoglobulin loop domain of immunoglobulin gene super-family member proteins may be used in whole or in part as alternative hinge region polypeptides as provided herein, or may be further modified for such use.

As noted above, the constructs of the invention, including binding domain-immunoglobulin fusion proteins, are believed, according to non-limiting theory, to be compromised in their ability to dimerize via interchain disulfide bond formation, and further according to theory, this property is a consequence, in whole or in part, of a reduction in the number of cysteine residues that are present in an immunoglobulin hinge region polypeptide selected for inclusion in the construction of the construct, such as a fusion protein construct. Determination of the relative ability of a polypeptide to dimerize is well within the knowledge of the relevant art, where any of a number of established methodologies may be applied to detect protein dimerization (see, *e.g.*, Scopes, *Protein Purification: Principles and Practice*, 1987 Springer-Verlag, New York). For example, biochemical separation techniques for resolving proteins on the basis of molecular size (*e.g.*, gel electrophoresis, gel filtration chromatography, analytical ultracentrifugation, *etc.*), and/or comparison of protein physicochemical properties before and after introduction of sulfhydryl-active (*e.g.*, iodoacetamide, N-ethylmaleimide) or disulfide-reducing (*e.g.*, 2-mercaptoethanol, dithiothreitol) agents, or other equivalent methodologies, may all be employed for determining a degree of polypeptide dimerization or oligomerization, and for determining possible contribution of disulfide bonds to such potential quaternary structure. In certain embodiments, the invention relates to a construct, for example a binding domain-immunoglobulin fusion protein, that exhibits a reduced (*i.e.*, in a statistically significant manner relative to an appropriate IgG-derived control, for example) ability to dimerize, relative to a wild-type human immunoglobulin G hinge region polypeptide as provided herein. Those familiar with the art will be able readily to determine whether a particular fusion protein displays such reduced ability to dimerize.

Compositions and methods for preparation of immunoglobulin fusion proteins, for example, are well known in the art. See, *e.g.*, U.S. Patent No. 5,892,019, which reports recombinant proteins that are the products of a single encoding polynucleotide but which are not constructs, including binding domain-immunoglobulin fusion proteins, according to the present invention.

For a construct, for example, an immunoglobulin fusion protein of the invention which is intended for use in humans, any included Ig constant regions will

typically be of human sequence origin, or humanized, to minimize a potential anti-human immune response and to provide appropriate and/or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In particularly preferred embodiments, a tail region is prepared from an immunoglobulin heavy chain constant region in which the CH1 domain is or has been deleted (the CH1 and CH2 regions in the case of IgE) and the carboxyl end of the binding domain, or where the binding domain comprises two immunoglobulin variable region polypeptides, the second (*i.e.*, more proximal to the C-terminus) variable region is joined to the amino terminus of CH2 through one or more connecting regions, such as a hinge or altered region. A schematic diagram depicting the structures of two exemplary binding domain-immunoglobulin fusion proteins is shown in FIG. 11. In particularly preferred embodiments no interchain disulfide bonds are present, and in other embodiments a restricted number of interchain disulfide bonds may be present relative to the number of such bonds that would be present if wild-type hinge region polypeptides were instead present. In other embodiments a construct of the invention, such as for example, a fusion protein, comprises, or consists essentially of, or consists of, a mutated or otherwise altered hinge region polypeptide that exhibits a reduced ability to dimerize, relative to a wild-type human IgG hinge region polypeptide. Thus, an isolated polynucleotide molecule coding for such a single chain construct, such as an immunoglobulin fusion protein, has a binding region, for example, a domain that provides specific or otherwise desired binding affinity and selectivity for a target, such as a target antigen.

The invention also contemplates, for example, in certain embodiments, constructs including binding domain-immunoglobulin fusion proteins that comprise fused or otherwise connected polypeptide sequences or portions thereof derived or prepared from a plurality of genetic sources, for example, according to molecular "domain swapping" paradigms. Those having familiarity with the art will appreciate that selection of such polypeptide sequences for assembly into a construct, such as a binding domain-immunoglobulin fusion protein, for example, may involve determination of appropriate portions of each component polypeptide sequence, for example, based on structural and/or

functional properties of each such sequence (see, *e.g.*, Carayannopoulos *et al.*, 1996 *J. Exp. Med.* **183**:1579; Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988)). The component polypeptide sequences of which the construct, such as a fusion protein, is comprised or prepared may therefore comprise  
5 intact or full-length binding domain, immunoglobulin, linker and/or plasma membrane anchor domain polypeptide sequences, or truncated versions or variants thereof such as those provided herein. According to these and related embodiments of the invention, any two or more of the candidate component polypeptides of which the subject invention constructs, for example, fusion proteins, may be comprised will be derived or prepared from  
10 independent sources, such as from immunoglobulin sequences of differing allotype, isotype, subclass, class, or species of origin (*e.g.*, xenotype). Thus, as a non-limiting example, a binding domain polypeptide (or its constituent polypeptides such as one or more variable region polypeptides and/or a linker polypeptide), a hinge region polypeptide, immunoglobulin heavy chain CH2 and CH3 constant region polypeptides and optionally an  
15 immunoglobulin heavy chain CH4 constant region polypeptide as may be obtained from an IgM or IgE heavy chain, and a plasma membrane anchor domain polypeptide may all be separately obtained from distinct genetic sources and engineered into a chimeric or fusion protein using well known techniques and according to methodologies described herein, for example.

20 Accordingly, a construct of the invention, for example a binding domain-immunoglobulin fusion protein according to certain embodiments of the present invention, may also therefore comprise in pertinent part an immunoglobulin heavy chain CH3 constant region polypeptide that is a wild-type IgA CH3 constant region polypeptide, or alternatively, that is a mutated or otherwise altered or substituted or truncated IgA CH3 constant region  
25 polypeptide that is incapable of associating with a J chain, or that will not associate to an undesired degree with a J chain; preferably the IgA CH3 constant region polypeptides used in a tail region portion of a construct are of human origin or are humanized. By way of brief background, IgA molecules are known to be released into secretory fluids by a mechanism that involves association of IgA into disulfide-linked polymers (*e.g.*, dimers) via a J chain  
30 polypeptide (*e.g.*, Genbank Acc. Nos. XM\_059628, M12378, M12759; Johansen *et al.*, 1999

*Eur. J. Immunol.* **29**:1701) and interaction of the complex so formed with another protein that acts as a receptor for polymeric immunoglobulin, and which is known as transmembrane secretory component (SC; Johansen *et al.*, 2000 *Sc. J. Immunol.* **52**:240; see also, *e.g.*, Sorensen *et al.*, 2000 *Int. Immunol.* **12**:19; Yoo *et al.*, 1999 *J. Biol. Chem.* **274**:33771; Yoo *et al.*, 2002 *J. Immunol. Meth.* **261**:1; Cortesy, 2002 *Trends Biotechnol.* **20**:65; Symersky *et al.*, 2000 *Mol. Immunol.* **37**:133; Crottet *et al.*, 1999 *Biochem. J.* **341**:299). Interchain disulfide bond formation between IgA Fc domains and J chain is mediated through a penultimate cysteine residue in an 18-amino acid C-terminal extension that forms part of the IgA heavy chain constant region CH3 domain polypeptide (Yoo *et al.*, 1999; Sorensen *et al.*, 2000). Certain embodiments of the subject invention constructs, including for example, fusion proteins, therefore contemplate inclusion of the wild-type IgA heavy chain constant region polypeptide sequence, which is capable of associating with J chain. Certain other embodiments of the invention, however, contemplate fusion proteins that comprise a mutated or otherwise altered, substituted, or truncated IgA CH3 constant region polypeptide that is incapable of associating with a J chain. According to such embodiments, for example, two or more residues from the C-terminus of an IgA CH3 constant region polypeptide such as a human IgA CH3 constant region polypeptide may be deleted to yield a truncated CH3 constant region polypeptide as provided herein. In preferred embodiments and as described in greater detail herein, a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain comprises such a C-terminal deletion of either four or 18 amino acids. However, the invention need not be so limited, such that the mutated IgA CH3 constant region polypeptide may comprise a deletion of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,, 21-25, 26-30 or more amino acids, so long as the construct, for example, the fusion protein, is capable of specifically binding an antigen and of at least one immunological activity as provided herein. Alternatively, the invention also contemplates constructs, for example, fusion proteins, having a tail region that comprises a mutated IgA CH3 constant region polypeptide that is incapable of associating with a J chain by virtue of replacement of the penultimate cysteine, or by chemical modification of that amino acid residue, in a manner that prevents, or inhibits an undesired level of, interchain disulfide bond or multimer formation. Methods



for determining whether a construct, for example a fusion protein, can associate with a J chain will be known to those having familiarity with the art and are described or referenced herein.

As also described herein and according to procedures known in the art, the construct, for example a fusion protein, may further be tested routinely for immunological activity, for instance, in ADCC or CDC assays. As an illustrative example, a construct, for example a fusion protein, according to such an embodiment may comprise a binding domain polypeptide derived or constructed from (or using) a native or engineered human heavy chain variable region polypeptide sequence, a native or engineered human IgA-derived immunoglobulin hinge region polypeptide sequence, a native or engineered human IgG1 immunoglobulin heavy chain CH2 constant region polypeptide sequence, a native or engineered human IgG2 immunoglobulin heavy chain CH3 constant region polypeptide sequence, and optionally a native or engineered human IgE immunoglobulin heavy chain CH4 constant region polypeptide sequence and/or a native or engineered human TNF- $\alpha$  receptor type 1 (TNFR1) plasma membrane anchor domain polypeptide sequence that comprises a cytoplasmic tail polypeptide which is capable of apoptotic signaling or otherwise promoting apoptosis. The invention therefore contemplates these and other embodiments according to the present invention in which two or more polypeptide sequences that are present in a construct, for example a fusion protein, have independent genetic origins.

As noted above, in certain embodiments the construct, for example a binding protein-immunoglobulin fusion protein, comprises at least one native or engineered immunoglobulin variable region polypeptide, which may be a native or engineered light chain or a native or engineered heavy chain variable region polypeptide, and in certain embodiments the fusion protein comprises at least one such native or engineered light chain V-region and one such native or engineered heavy chain V-region and at least one linker peptide that is fused or otherwise connected to to each of the native or engineered V-regions. Construction of such binding domains, for example single chain Fv domains, is known in the art and is described in greater detail in the Examples below, and has been described, for example, in various documents cited herein; selection and assembly of single-chain variable

regions and of linker polypeptides that may be fused or otherwise connected to each of a heavy chain-derived and a light chain-derived V region (*e.g.*, to generate a binding region, such as a binding domain that comprises a single-chain Fv polypeptide) is also known to the art and described herein. See, *e.g.*, U.S. Patent Nos. 5,869,620, 4,704,692, and 4,946,778.

5 In certain embodiments all or a portion or portions of an immunoglobulin sequence that is derived from a non-human source may be “humanized” according to recognized procedures for generating humanized antibodies, *i.e.*, immunoglobulin sequences into which human Ig sequences are introduced to reduce the degree to which a human immune system would perceive such proteins as foreign (see, *e.g.*, U.S. Patent Nos. 5,693,762; 5,585,089; 10 4,816,567; 5,225,539; 5,530,101; and documents cited therein).

Constructs of the invention, including binding domain-immunoglobulin fusion proteins, as described herein may, according to certain embodiments, desirably comprise sites for glycosylation, *e.g.*, covalent attachment of carbohydrate moieties such as, for example, monosaccharides or oligosaccharides. Incorporation of amino acid sequences that provide substrates for polypeptide glycosylation is within the scope of the relevant art, 15 including, for example, the use of genetic engineering or protein engineering methodologies to obtain a polypeptide sequence containing, for example, the classic Asn-X-Ser/Thr site for N-(asparagine)-linked glycosylation, or a sequence containing Ser or Thr residues that are suitable substrates for O-linked glycosylation, or sequences amenable to C-mannosylation, 20 glypiation/ glycosylphosphatidylinositol modification, or phosphoglycation, all of which can be identified according to art-established criteria (*e.g.*, Spiro, 2002 *Glybiology* 12:43R). Without wishing to be bound by any particular theory or mechanism, glycosylated constructs such as fusion proteins having particular amino acid sequences may beneficially possess attributes associated with one or more of improved solubility, enhanced stability in 25 solution, enhanced physiological stability, improved bioavailability including *in vivo* biodistribution, and superior resistance to proteases, all in a statistically significant manner, relative to constructs, including fusion proteins, having the same or highly similar amino acid sequences but lacking glycosyl moieties. In certain preferred embodiments the subject invention constructs, such as fusion protein constructs, may comprise a glycosylation site 30 that is present in a linker as provided herein, and in certain other preferred embodiments the

subject invention construct, for example, a fusion protein, comprises a glycosylation site that is present in a connecting region, such as a hinge region polypeptide sequence as provided herein.

5 In certain preferred embodiments of the present invention, such as those useful for gene therapy applications or in display systems or assays, such as screening assays (including library display systems and library screening assays), the construct, for example, a binding domain-immunoglobulin fusion protein, is a protein or glycoprotein that is capable of being expressed by a host cell such that it localizes to the cell surface. Constructs, such as binding domain-immunoglobulin fusion proteins, that localize to the cell surface may do  
10 so by virtue of having naturally present or artificially introduced structural features that direct the fusion protein to the cell surface (e.g., Nelson *et al.* 2001 *Trends Cell Biol.* **11**:483; Ammon *et al.*, 2002 *Arch. Physiol. Biochem.* **110**:137; Kasai *et al.*, 2001 *J. Cell Sci.* **114**:3115; Watson *et al.*, 2001 *Am. J. Physiol. Cell Physiol.* **281**:C215; Chatterjee *et al.*, 200 *J. Biol. Chem.* **275**:24013) including by way of illustration and not limitation, secretory  
15 signal sequences, leader sequences, plasma membrane anchor domain polypeptides and transmembrane domains such as hydrophobic transmembrane domains (e.g., Heuck *et al.*, 2002 *Cell Biochem. Biophys.* **36**:89; Sadlish *et al.*, 2002 *Biochem J.* **364**:777; Phoenix *et al.*, 2002 *Mol. Membr. Biol.* **19**:1; Minke *et al.*, 2002 *Physiol. Rev.* **82**:429) or glycosylphosphatidylinositol attachment sites (“glypiation” sites, e.g., Chatterjee *et al.*, 2001  
20 *Cell Mol. Life Sci.* **58**:1969; Hooper, 2001 *Proteomics* **1**:748; Spiro, 2002 *Glycobiol.* **12**:43R), cell surface receptor binding domains, extracellular matrix binding domains, or any other structural feature that causes at least a desired portion of the fusion protein population to localize, in whole or in part, to the cell surface. Particularly preferred are fusion protein constructs that comprise a plasma membrane anchor domain which includes a  
25 transmembrane polypeptide domain, typically comprising a membrane spanning domain which includes a hydrophobic region capable of energetically favorable interaction with the phospholipid fatty acyl tails that form the interior of the plasma membrane bilayer. Such features are known to those of ordinary skill in the art, who will further be familiar with methods for introducing nucleic acid sequences encoding these features into the subject

expression constructs by genetic engineering, and with routine testing of such constructs to verify cell surface localization of the product.

According to certain further embodiments, a plasma membrane anchor domain polypeptide comprises such a transmembrane domain polypeptide and also comprises a cytoplasmic tail polypeptide, which refers to a region or portion of the polypeptide sequence that contacts the cytoplasmic face of the plasma membrane and/or is in contact with the cytosol or other cytoplasmic components. A large number of cytoplasmic tail polypeptides are known that comprise the intracellular portions of plasma membrane transmembrane proteins, and discrete functions have been identified for many such polypeptides, including biological signal transduction (*e.g.*, activation or inhibition of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides and other second messengers, ion channels, secretory pathways), biologically active mediator release, stable or dynamic association with one or more cytoskeletal components, cellular differentiation, cellular activation, mitogenesis, cytostasis, apoptosis and the like (*e.g.*, Maher *et al.*, 2002 *Immunol. Cell Biol.* **80**:131; El Far *et al.*, 2002 *Biochem J.* **365**:329; Teng *et al.*, 2002 *Genome Biol.* 2REVIEWS:3012; Simons *et al.*, 2001 *Cell Signal* **13**:855; Furie *et al.*, 2001 *Thromb. Haemost.* **86**:214; Gaffen, 2001 *Cytokine* **14**:63; Dittel, 2000 *Arch. Immunol. Ther. Exp.* (Warsz.) **48**:381; Parnes *et al.*, 2000 *Immunol. Rev.* **176**:75; Moretta *et al.*, 2000 *Semin. Immunol.* **12**:129; Ben Ze'ev, 1999 *Ann. N.Y. Acad. Sci.* **886**:37; Marsters *et al.*, *Recent Prog. Horm. Res.* **54**:225).

Figure 70 illustrates the binding, for example, of fluorocaine-conjugated FcRIII (CD16) soluble fusion proteins to 2H7 scFv-binding domain constructs that are attached to CD20 expressed by cells, CHO cells in this example. CD16 binding to a construct of the invention, for example, scFv-binding domain construct, provides one example of a screening tool that may be used to detect and/or quantitate changes in CD16 binding to altered constructs of the invention, including scFv-binding domain constructs, that contains targeted or site-specific mutations, substitutions, deletions, or other alterations. Changes in CD16 binding properties may be reflected, for example, by changes in binding of either CD16 high affinity protein (158V) or CD16 low affinity protein (158 F) or both.

A schematic representation of one example of such a screening process is diagrammed in the second drawing in Figure 70, in which scFv-binding domain constructs are displayed on the cell surface of mammalian cells. scFv-binding domain molecules in this example are displayed on the cell surface through a molecule that serves as a transmembrane domain anchor. These molecules may represent, for example, a single scFv-binding domain construct or may be introduced into a population of mammalian cells as a library of such molecules. Transfected cells with altered binding properties can then, for example, be panned, sorted, or otherwise isolated from other cells by altering the stringency of the selection conditions and using CD16 fusion proteins as a binding probe. Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158V) or CD16 low affinity allele (158F) or both, for example, can be isolated.

This display system can be used, for example, to create a library of constructs of the invention with mutated or otherwise altered tail regions with short stretches of CH2 sequence replaced with randomized oligonucleotides or, for example, randomization of a single residue with all possible amino acid substitutions, natural or unnatural, including synthetic amino acids. Once such a library is constructed, it can be transfected into appropriate cells, for example, COS cells, by methods known in the art. Transfectants can then be bound to, for example, labeled CD16 constructs, and panned or sorted based on their relative or desired binding properties to multiple allelotypes/isoforms. Desired cells may be harvested, and the DNA, for example, plasmid DNA, isolated and then transformed into, for example, bacteria. This process may be repeated iteratively multiple times until desired single clones are isolated from the mammalian host cells. See Seed B and Aruffo A, *Pro. Nat'l Acad Sci USA* 1987 **84**:3365-3369; Aruffo A and Seed B, *Pro. Nat'l Acad Sci USA* 1987 **84**:8573-8577.

One such use of this type of screening system, for example, is for the identification and/or isolation of constructs of the invention having tail regions, or tail regions, that bind equally well to both the high and low affinity alleles of CD16 with the goal of improving effector functions mediated by scFv-binding domain constructs in multiple subpopulations of patients. Constructs of the invention having tail regions, or tail regions with altered binding properties to other Fc receptors can also be selected using such

a display system, for example, the display system described. Other display systems that do not glycosylate proteins, for example, those that use bacteriophage or yeast, are not generally desired for selection of constructs of the invention having Ig-based tail regions, or Ig-based tail regions, with altered FcR binding properties. Most non-mammalian systems, for example, do not glycosylate proteins.

Expression of constructs of the invention, for example, scFv-binding domain constructs, expressed on the surface of a mammalian cell by incorporation of an appropriate molecule into the construct, for example, by incorporation of a transmembrane domain or a GPI anchor signal, also have utility in other display systems that are useful, for example, for selection of constructs of the invention, for example, altered scFv-binding domain molecules that will be produced at higher or other desired levels. In one such an embodiment, cells that are useful in the production of glycosylated proteins, for example, mammalian cells such as COS cells, are transfected with a library of scFv-binding domain constructs in a plasmid that directs their expression to the cell surface. Cells, such as COS cells, that express the highest or other desired level of the scFv-binding domain molecules are selected by techniques known in the art (for example panning, sterile cell sorting, magnetic bead separation, *etc.*), and DNA, for example, plasmid DNA, is isolated for transformation into other cells, for example, bacteria. After one or more rounds of selection single clones are isolated that encode scFv-binding domain molecules capable of a high or other desired level of expression. The isolated clones may then be altered to remove the membrane anchor and expressed in an appropriate cells system, for example, a mammalian cell system, wherein the scFv-binding domain constructs will be produced, for example, by secretion into the culture fluid at desired levels. Without being bound by any particular mechanism or theory, this is believed to result from the common requirement of secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression. Thus, selection for a molecule that shows an improvement expression levels on a cell surface will also result in the identification of a molecule having an improvement in levels of secreted protein.

These display systems utilizing a construct of the invention may also be used for screening and/or identifying and/or isolating affinity variants of the binding domain within a construct.

Particularly preferred are such display and/or screening systems, for example, that include or use constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered  $V_H$  and/or  $V_L$  and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a  $V_H$  region sequence (including in a  $V_H$  region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered  $V_H$  and/or  $V_L$  and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a  $V_L$  region sequence within an scFv or other construct). Especially preferred are such display and/or screening systems that include or use constructs that include an engineered  $V_H$  sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11. The  $V_H$ 11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

In the context of other methods of using constructs of the invention, including binding domain-immunoglobulin fusion proteins, for the treatment of a malignant condition or a B cell disorder(s) as provided herein, including, for example, by one or more of a number of gene therapy methods and related construct delivery techniques, the present invention also contemplates certain embodiments wherein a construct, for example, a binding domain-immunoglobulin fusion protein that comprises a plasma membrane anchor domain polypeptide is expressed (or capable of expression) at a cell surface and may further comprise a cytoplasmic tail polypeptide which comprises an apoptosis signaling polypeptide

sequence. A number of apoptosis signaling polypeptide sequences are known to the art, as reviewed, for example, in *When Cells Die: A Comprehensive Evaluation of Apoptosis and Programmed Cell Death* (R.A. Lockshin *et al.*, Eds., 1998 John Wiley & Sons, New York; see also, *e.g.*, Green *et al.*, 1998 *Science* **281**:1309 and references cited therein; Ferreira *et al.*, 2002 *Clin. Canc. Res.* **8**:2024; Gurumurthy *et al.*, 2001 *Cancer Metastas. Rev.* **20**:225; Kanduc *et al.*, 2002 *Int. J. Oncol.* **21**:165). Typically an apoptosis signaling polypeptide sequence comprises all or a portion of, or is derived or constructed from, a receptor death domain polypeptide, for instance, FADD (*e.g.*, Genbank Acc. Nos. U24231, U43184, AF009616, AF009617, NM\_012115), TRADD (*e.g.*, Genbank Acc. No. NM\_003789), RAIDD (*e.g.*, Genbank Acc. No. U87229), CD95 (FAS/Apo-1; *e.g.*, Genbank Acc. Nos. X89101, NM\_003824, AF344850, AF344856), TNF- $\alpha$ -receptor-1 (TNFR1, *e.g.*, Genbank Acc. Nos. S63368, AF040257), DR5 (*e.g.*, Genbank Acc. No. AF020501, AF016268, AF012535), an ITIM domain (*e.g.*, Genbank Acc. Nos. AF081675, BC015731, NM\_006840, NM\_006844, NM\_006847, XM\_017977; see, *e.g.*, Billadeau *et al.*, 2002 *J. Clin. Invest.* **109**:161), an ITAM domain (*e.g.*, Genbank Acc. Nos. NM\_005843, NM\_003473, BC030586; see, *e.g.*, Billadeau *et al.*, 2002), or other apoptosis-associated receptor death domain polypeptides known to the art, for example, TNFR2 (*e.g.*, Genbank Acc. No. L49431, L49432), caspase/procaspase-3 (*e.g.*, Genbank Acc. No. XM\_54686), caspase/procaspase-8 (*e.g.*, AF380342, NM\_004208, NM\_001228, NM\_033355, NM\_033356, NM\_033357, NM\_033358), caspase/procaspase-2 (*e.g.*, Genbank Acc. No. AF314174, AF314175), *etc.*

Cells in a biological sample that are suspected of undergoing apoptosis may be examined for morphological, permeability or other changes that are indicative of an apoptotic state. For example by way of illustration and not limitation, apoptosis in many cell types may cause altered morphological appearance such as plasma membrane blebbing, cell shape change, loss of substrate adhesion properties or other morphological changes that can be readily detected by a person having ordinary skill in the art, for example by using light microscopy. As another example, cells undergoing apoptosis may exhibit fragmentation and disintegration of chromosomes, which may be apparent by microscopy and/or through the use of DNA-specific or chromatin-specific dyes that are known in the art,



including fluorescent dyes. Such cells may also exhibit altered plasma membrane permeability properties as may be readily detected through the use of vital dyes (e.g., propidium iodide, trypan blue) or by the detection of lactate dehydrogenase leakage into the extracellular milieu. These and other means for detecting apoptotic cells by morphologic  
5 criteria, altered plasma membrane permeability, and related changes will be apparent to those familiar with the art.

In another embodiment of the invention wherein a construct, such as a binding domain-immunoglobulin fusion protein, that is expressed at a cell surface comprises a plasma membrane anchor domain having a transmembrane domain and a cytoplasmic tail  
10 that comprises an apoptosis signaling polypeptide, cells in a biological sample may be assayed for translocation of cell membrane phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, which may be detected, for example, by measuring outer leaflet binding by the PS-specific protein annexin. Martin *et al.*, *J. Exp. Med.* **182**:1545, 1995; Fadok *et al.*, *J. Immunol.* **148**:2207, 1992. In still other related  
15 embodiments of the invention, including embodiments wherein a construct, such as a binding domain-immunoglobulin fusion protein, is expressed at the cell surface and comprises a plasma membrane anchor domain having an apoptosis signaling polypeptide and also including embodiments wherein the construct, such as a binding domain-immunoglobulin fusion protein, is a soluble protein that lacks a membrane anchor domain  
20 and that is capable of inducing apoptosis, a cellular response to an apoptogen is determined by an assay for induction of specific protease activity in any member of a family of apoptosis-activated proteases known as the caspases (see, e.g., Green *et al.*, 1998 *Science* **281**:1309). Those having ordinary skill in the art will be readily familiar with methods for determining caspase activity, for example by determination of caspase-mediated cleavage of  
25 specifically recognized protein substrates. These substrates may include, for example, poly-(ADP-ribose) polymerase (PARP) or other naturally occurring or synthetic peptides and proteins cleaved by caspases that are known in the art (see, e.g., Ellerby *et al.*, 1997 *J. Neurosci.* **17**:6165). The synthetic peptide Z-Tyr-Val-Ala-Asp-AFC (SEQ ID NO:\_\_), wherein "Z" indicates a benzoyl carbonyl moiety and AFC indicates 7-amino-4-  
30 trifluoromethylcoumarin (Kluck *et al.*, 1997 *Science* **275**:1132; Nicholson *et al.*, 1995

*Nature* 376:37), is one such substrate. Other non-limiting examples of substrates include nuclear proteins such as U1-70 kDa and DNA-PKcs (Rosen and Casciola-Rosen, 1997 *J. Cell. Biochem.* 64:50; Cohen, 1997 *Biochem. J.* 326:1). Cellular apoptosis may also be detected by determination of cytochrome c that has escaped from mitochondria in apoptotic cells (*e.g.*, Liu *et al.*, *Cell* 86:147, 1996). Such detection of cytochrome c may be performed spectrophotometrically, immunochemically or by other well established methods for determining the presence of a specific protein. Persons having ordinary skill in the art will readily appreciate that there may be other suitable techniques for quantifying apoptosis.

Particularly preferred embodiments of constructs useful for gene therapy applications, including those constructs that include a plasma membrane anchor domain and/or cytoplasmic tail polypeptide (including, for example, an apoptosis signaling sequence), are such constructs that include (1) an immunoglobulin variable region polypeptide sequence, including native or engineered V<sub>H</sub> and/or V<sub>L</sub> and/or single-chain variable region (sFv) sequences, and which include, for example, a mutation, alteration or deletion at an amino acid at a location or locations corresponding to one or more of amino acid positions 9, 10, 11, 12, 108, 110, 111, and 112, in a V<sub>H</sub> region sequence (including in a V<sub>H</sub> region sequence within an scFv or other construct), and/or (2) an immunoglobulin variable region polypeptide sequence, including native or engineered V<sub>H</sub> and/or V<sub>L</sub> and/or single-chain variable region (sFv) sequences, and which include, for example, a a mutation, alteration or deletion at a location or locations corresponding to one or more of amino acid positions 12, 80, 81, 82, 83, 105, 106, 107 and 108 in a light chain variable region sequence (including in a V<sub>L</sub> region sequence within an scFv or other construct). Especially preferred are constructs that include an engineered V<sub>H</sub> sequence (whether or not associated with one or more other sequences, including immunoglobulin-derived and other sequences contained, for example, within an sFv or scFv-containing construct), which includes a mutation, alteration or deletion at an amino acid at a location or locations corresponding to amino acid position 11. The V<sub>H</sub>11 amino acid, if substituted, may be substituted with another amino acid as described herein, or by another molecule as desired.

Once a construct, such as for example a binding domain-immunoglobulin fusion protein, as provided herein has been designed, polynucleotides including DNAs

encoding the construct, where it or a relevant portion of it is a polypeptide, may be synthesized in whole or in part via oligonucleotide synthesis as described, for example, in Sinha *et al.*, *Nucleic Acids Res.*, **12**:4539-4557 (1984); assembled via PCR as described, for example in Innis, Ed., *PCR Protocols*, Academic Press (1990) and also in Better *et al.* *J. Biol. Chem.* **267**:16712-16118 (1992); cloned and expressed via standard procedures as described, for example, in Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989) and also in Robinson *et al.*, *Hum. Antibod. Hybridomas*, **2**:84-93 (1991); and tested for desired activity, for example, binding to a target, or specific antigen binding activity, as described, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988) and Munson *et al.*, *Anal. Biochem.*, **107**:220-239 (1980).

The preparation of single polypeptide chain binding molecules of the Fv region, single-chain Fv molecules, is known in the art. See, *e.g.*, U.S. Pat. No. 4,946,778. In the present invention, single-chain Fv-like molecules that may be included in constructs of the invention may be synthesized by encoding a first variable region of the heavy or light chain, followed by one or more linkers to the variable region of the corresponding light or heavy chain, respectively. The selection of various appropriate linker(s) between the two variable regions is described in U.S. Pat. No. 4,946,778 (see also, *e.g.*, Huston *et al.*, 1993 *Int. Rev. Immunol.* **10**:195). An exemplary linker described herein is (Gly-Gly-Gly-Gly-Ser)<sub>3</sub>, but may be of any desired length. The linker is used to convert the naturally aggregated but chemically separate heavy and light chains into the amino terminal antigen binding portion of a single polypeptide chain, for example, wherein this antigen binding portion will fold into a structure similar to the original structure made of two polypeptide chains, or that otherwise has the ability to bind to a target, for example a target antigen. For those constructs that include an scFv as a binding region, a native or engineered immunoglobulin hinge as a connecting region, and one or more native or engineered heavy chain constant regions as a binding region, nucleotide sequences encoding the variable regions of native or engineered heavy and light chains, joined by a sequence encoding a linker, are joined to a nucleotide sequence encoding native or engineered antibody constant regions, as desired. The constant regions may be those that permit the resulting polypeptide

to form interchain disulfide bonds to form a dimer, and which contain desired effector functions, such as the ability to mediate ADCC, CDC, or fix complement, although native or engineered constant regions that do not favor dimer or other multimer formation or aggregation are preferred.. For a construct, such as an immunoglobulin-like molecule, of the invention that is intended for use in humans, the included sequences having constant regions and/or desired constant regions function(s) will typically be human or substantially human or humanized to minimize a potential anti-human immune response and to provide appropriate or desired effector functions. Manipulation of sequences encoding antibody constant regions is referenced in the PCT publication of Morrison and Oi, WO 89/07142. In preferred embodiments, the CH1 domain is deleted in whole or in part from a tail region that includes, or consists essentially of, or consists of, a native or engineered immunoglobulin constant region(s) (for example, native or engineered CH2 and/or CH3 constant region(s), or native or engineered CH2 and/or CH3 and/or CH4 constant region(s)), and the carboxyl end of the binding region, for example, a binding domain polypeptide such as an immunoglobulin variable region polypeptide, is joined to the amino terminus of, for example, a CH2 via a connecting region, for example, a native or engineered hinge region polypeptide as provided herein.

As described above, the present invention provides recombinant expression constructs capable of directing the expression of constructs of the invention, including binding domain-immunoglobulin fusion proteins, as provided herein. The amino acids, which occur in the various amino acid sequences referred to herein, are identified according to their well known three-letter or single-letter abbreviations. The nucleotides, which occur in the various DNA sequences or fragments thereof referred herein, are designated with the standard single letter designations used routinely in the art. A given amino acid sequence may also encompass similar but changed amino acid sequences, such as those having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include conservative substitutions or substitutions with non-naturally-occurring amino acids. Amino acid sequences that are similar to one another may share substantial regions of sequence homology. In like fashion, nucleotide sequences may encompass substantially similar

nucleotide sequences having only minor changes, for example by way of illustration and not limitation, covalent chemical modifications, insertions, deletions and substitutions, which may further include silent mutations owing to degeneracy of the genetic code. Nucleotide sequences that are similar to one another may share substantial regions of sequence  
5 homology.

The presence of a malignant condition in a subject refers to the presence of dysplastic, cancerous, and/or transformed cells in the subject, including, for example, neoplastic, tumor, non-contact inhibited, or oncogenically transformed cells, or the like (*e.g.*, melanoma, carcinomas such as adenocarcinoma, squamous cell carcinoma, small cell  
10 carcinoma, oat cell carcinoma, *etc.*, sarcomas such as chondrosarcoma, osteosarcoma, *etc.*) which are known to the art and for which criteria for diagnosis and classification are established. In preferred embodiments contemplated by the present invention, for example, such cancer cells are malignant hematopoietic cells, such as transformed cells of lymphoid lineage and in particular, B cell lymphomas and the like; cancer cells may in certain  
15 preferred embodiments also be epithelial cells such as carcinoma cells. The invention also contemplates B cell disorders, which may include certain malignant conditions that affect B cells (*e.g.*, B cell lymphoma) but which is not intended to be so limited, and which is also intended to encompass autoimmune diseases and in particular, diseases, disorders and conditions that are characterized by autoantibody production, for example.

20 Autoantibodies are antibodies that react with self antigens. Autoantibodies are detected in several autoimmune diseases (*i.e.*, a disease, disorder or condition wherein a host immune system generates an inappropriate anti-“self” immune reaction) where they are involved in disease activity. Current treatments for various autoimmune diseases include immunosuppressive drugs that require continuing administration, lack specificity, and cause  
25 significant side effects. New approaches that can eliminate autoantibody production with minimal toxicity will address an unmet medical need for a spectrum of diseases that affect many people. Constructs of the subject invention, including binding domain-immunoglobulin fusion proteins, are designed, for example, for improved penetration into lymphoid tissues. Depletion of B lymphocytes interrupts the autoantibody production cycle,

and allows the immune system to reset as new B lymphocytes are produced from precursors in the bone marrow.

A number of diseases, disorders, and conditions have been identified for which beneficial effects are believed, according to non-limiting theory, to result from B cell depletion therapy. Such diseases disorders, and conditions include, but are not limited to, Grave's disease, Hashimoto's disease, rheumatoid arthritis, systemic lupus erythematosus, Sjogrens Syndrome Immune Thrombocytopenic purpura, multiple sclerosis, myasthenia gravis, scleroderma, psoriasis, Inflammatory Bowel Disease including Crohn's disease and ulcerative colitis, .. Inflammatory Bowel Disease including Crohn's disease and Ulcerative colitis, are autoimmune diseases of the digestive system.

The present invention further relates to nucleotide constructs encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, and in particular to methods for administering recombinant constructs encoding such proteins for gene therapy applications that may be expressed, for example, as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to constructs of the invention including, for example, binding domain-immunoglobulin fusion polypeptides or fusion proteins, refers to any construct, such as a binding domain-immunoglobulin fusion polypeptide or fusion protein, that retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a pro- or prepro- form of a construct, for example, a pro-protein that can be activated by cleavage of the pro-protein portion to produce an active construct, such as a binding domain-immunoglobulin fusion polypeptide.

A fragment, derivative or analog of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide or fusion protein, including binding domain-immunoglobulin fusion polypeptides or fusion proteins encoded by the cDNAs referred to herein, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a

substituent group, or (iii) one in which additional amino acids are fused or otherwise connected to the construct, *e.g.*, a binding domain-immunoglobulin fusion polypeptide, including amino acids that are employed for detection or specific functional alteration of the construct, including such constructs as a binding domain-immunoglobulin fusion polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The constructs, including polypeptide constructs, of the present invention include, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins having binding regions such as binding domain polypeptide amino acid sequences that are identical or similar to sequences known in the art, or fragments or portions thereof. For example by way of additional illustration and not limitation, a human CD154 molecule extracellular domain [SEQ ID NO:\_\_\_] is contemplated for use according to the instant invention, as are portions of such polypeptides and/or polypeptides having at least about 70% similarity (preferably greater than a 70% identity) and more preferably about 90% similarity (more preferably greater than a 90% identity) to the reported polypeptide and still more preferably about 95% similarity (still more preferably greater than a 95% identity) to the reported polypeptides and to portions of such polypeptides, wherein such portions of a binding domain-immunoglobulin fusion polypeptide, for example, generally contain at least about 30 amino acids and more preferably at least about 50 amino acids. Extracellular domains include, for example, portions of a cell surface molecule, and in particularly preferred embodiments cell surface molecules that are integral membrane proteins or that comprise a plasma membrane spanning transmembrane domain, that are constructed to extend beyond the outer leaflet of the plasma membrane phospholipid bilayer when the molecule is expressed at a cell surface, preferably in a manner that exposes the extracellular domain portion of such a molecule to the external environment of the cell, also known as the extracellular milieu. Methods for determining whether a portion of a cell surface molecule comprises an extracellular domain are well known to the art and include, for example, experimental determination (*e.g.*, direct or indirect labeling of the molecule, evaluation of whether the molecule can be structurally altered by agents to which the plasma membrane is not permeable such as proteolytic or lipolytic enzymes) or topological prediction based on

the structure of the molecule (*e.g.*, analysis of the amino acid sequence of a polypeptide) or other methodologies.

As used herein, an “amino acid” is a molecule having the structure wherein a central carbon atom (the alpha ( $\alpha$ )-carbon atom) is linked to a hydrogen atom, a carboxylic acid group (the carbon atom of which is referred to herein as a “carboxyl carbon atom”), an amino group (the nitrogen atom of which is referred to herein as an “amino nitrogen atom”), and a side chain group, R. When incorporated into a peptide, polypeptide, or protein, an amino acid loses one or more atoms of its amino and carboxylic groups in the dehydration reaction that links one amino acid to another. As a result, when incorporated into a protein, an amino acid may also be referred to as an “amino acid residue.” In the case of naturally occurring proteins, an amino acid residue’s R group differentiates the 20 amino acids from which proteins are typically synthesized, although one or more amino acid residues in a protein may be derivatized or modified following incorporation into protein in biological systems (*e.g.*, by glycosylation and/or by the formation of cystine through the oxidation of the thiol side chains of two non-adjacent cysteine amino acid residues, resulting in a disulfide covalent bond that frequently plays an important role in stabilizing the folded conformation of a protein, *etc.*). As those in the art will appreciate, non-naturally occurring amino acids can also be incorporated into proteins, particularly those produced by synthetic methods, including solid state and other automated synthesis methods. Examples of such amino acids include, without limitation,  $\alpha$ -amino isobutyric acid, 4-amino butyric acid, L-amino butyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteine, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids (*e.g.*,  $\beta$ -methyl amino acids,  $\alpha$ -methyl amino acids, N $\alpha$ -methyl amino acids) and amino acid analogs in general. In addition, when an  $\alpha$ -carbon atom has four different groups (as is the case with the 20 amino acids used by biological systems to synthesize proteins, except for glycine, which has two hydrogen atoms bonded to the  $\alpha$  carbon atom), two different enantiomeric forms of each amino acid exist, designated D and L. In mammals, only L-amino acids are incorporated into naturally occurring polypeptides.



The instant invention envisions proteins incorporating one or more D- and L- amino acids, as well as proteins comprised of just D- or L- amino acid residues.

Herein, the following abbreviations may be used for the following amino acids (and residues thereof): alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N);  
5 aspartic acid (Asp, D); cyteine (Cys, C); glycine (Gly, G); glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V). Non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan,  
10 and methionines. Neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, esparagine, and glutamine. Positively charged (basic amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

“Protein” refers to any polymer of two or more individual amino acids  
15 (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl carbon atom of the carboxylic acid group bonded to the  $\alpha$ -carbon of one amino acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the  $\alpha$ -carbon of an adjacent amino acid. The term “protein” is understood to include the terms “polypeptide” and “peptide” (which, at times, may be used  
20 interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits or other components will also be understood to be included within the meaning of “protein” as used herein. Similarly, fragments of proteins, peptides, and polypeptides are also within the scope of the invention and may be referred to herein as “proteins.”

25 In biological systems (be they *in vivo* or *in vitro*, including cell-free, systems), the particular amino acid sequence of a given protein (*i.e.*, the polypeptide’s “primary structure,” when written from the amino-terminus to carboxy-terminus) is determined by the nucleotide sequence of the coding portion of a mRNA, which is in turn specified by genetic information, typically genomic DNA (which, for purposes of this  
30 invention, is understood to include organelle DNA, for example, mitochondrial DNA and

chloroplast DNA). Of course, any type of nucleic acid which constitutes the genome of a particular organism (*e.g.*, double-stranded DNA in the case of most animals and plants, single or double-stranded RNA in the case of some viruses, *etc.*) is understood to code for the gene product(s) of the particular organism. Messenger RNA is translated on a ribosome, which catalyzes the polymerization of a free amino acid, the particular identity of which is specified by the particular codon (with respect to mRNA, three adjacent A, G, C, or U ribonucleotides in the mRNA's coding region) of the mRNA then being translated, to a nascent polypeptide. Recombinant DNA techniques have enabled the large-scale synthesis of proteins and polypeptides (*e.g.*, human insulin, human growth hormone, erythropoietin, granulocyte colony stimulating factor, *etc.*) having the same primary sequence as when produced naturally in living organisms. In addition, such technology has allowed the synthesis of analogs of these and other proteins, which analogs may contain one or more amino acid deletions, insertions, and/or substitutions as compared to the native proteins. Recombinant DNA technology also enables the synthesis of entirely novel proteins.

In non-biological systems (*e.g.*, those employing solid state synthesis), the primary structure of a protein (which also includes disulfide (cystine) bond locations) can be determined by the user. As a result, polypeptides having a primary structure that duplicates that of a biologically produced protein can be achieved, as can analogs of such proteins. In addition, completely novel polypeptides can also be synthesized, as can protein incorporating non-naturally occurring amino acids.

As is known in the art, "similarity" between two polypeptides may be determined by comparing the amino acid sequence (including conserved amino acid substitutes therein) of one polypeptide to the sequence of a second polypeptide. Fragments or portions of the nucleic acids encoding polypeptides of the present invention may be used to synthesize full-length nucleic acids of the present invention. As used herein, "% identity" refers to the percentage of identical amino acids situated at corresponding amino acid residue positions when two or more polypeptide are aligned and their sequences analyzed using, for example, a gapped BLAST algorithm (*e.g.*, Altschul *et al.*, 1997 *Nucl. Ac. Res.* **25**:3389; Altschul *et al.*, 1990 *J. Mol. Biol.*, **215**:403-410) which weights sequence gaps and sequence mismatches according to the default weightings provided by the National Institutes

of Health/ NCBI database (Bethesda, MD; *see* [www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast](http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast)). Other alignment methods include BLITZ (MPsrch) (Sturrock & Collins, 1993), and FASTA (Pearson and Lipman, 1988 *Proc. Natl. Acad. Sci. USA.* **85**:2444-2448).

The term “isolated” means, in the case of a naturally occurring material, that the material is or has been removed from, or is no longer associated with, its natural or original environment. For example, a naturally occurring nucleic acid or protein or polypeptide present in a living animal is not isolated, but the same nucleic acid or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such nucleic acids could be part of a vector and/or such nucleic acids or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The term “isolated”, in the case of non-naturally occurring material, such as a recombinantly manufactured construct of the invention, includes material that is substantially or essentially free from components which normally accompany it during manufacture, such as, for example, proteins and peptides that have been purified to a desired degree, preferably, for example, so that they are at least about 80% pure, more preferably at least about 90%, and still more preferably at least about 95% as measured by techniques known in the art.

The term “gene” means a segment of DNA involved in producing a polypeptide chain; it may also include regions preceding and following a polypeptide coding region, for example, a “leader and trailer” as well as intervening sequences (introns) between relevant individual coding segments (exons).

As described herein, the invention provides constructs, including binding domain-immunoglobulin fusion proteins, that may be encoded in whole or in part by nucleic acids that have a binding region coding sequence such as, for example, a binding domain coding sequence fused or otherwise connected in frame to an additional native or engineered immunoglobulin domain encoding sequence to provide for expression of, for example, a binding domain polypeptide sequence fused or otherwise connected to an additional functional polypeptide sequence that permits, for example by way of illustration and not limitation, detection, functional alteration, isolation and/or purification of the fusion protein. Such fusion proteins may permit functional alteration of a binding domain by containing

additional immunoglobulin-derived polypeptide sequences that influence behavior of the fusion product, for example (and as described above) by reducing the availability of sulfhydryl groups for participation in disulfide bond formation, and by conferring the ability to potentiate ADCC and/or CDC and/or fix complement.

5                   Modification of a polypeptide may be effected by any means known to those of skill in this art. The preferred methods herein rely on modification of DNA encoding, for example, a fusion protein and expression of the modified DNA. DNA encoding one of the constructs of the invention, for example, one of the binding domain-immunoglobulin fusions discussed herein, for example, may be altered or mutagenized using standard methodologies,  
10 including those described below. For example, cysteine residues that may otherwise facilitate multimer formation or promote particular molecular conformations can be deleted from a polypeptide or replaced, *e.g.*, cysteine residues that are responsible for or participate in aggregate formation. If necessary, for example, the identity of cysteine residues that contribute to aggregate formation may be determined empirically, by deleting and/or  
15 replacing a cysteine residue and ascertaining whether the resulting protein aggregates in solutions containing physiologically acceptable buffers and salts. In addition, fragments of, for example, binding domain-immunoglobulin fusions may be constructed and used. As noted above, counterreceptor/ligand binding domains for many candidate binding domain-immunoglobulin fusion have been delineated, such that one having ordinary skill in the art may readily select appropriate polypeptide domains for inclusion in encoded products of the  
20 instant expression constructs.

Conservative substitutions of amino acids are well-known and may be made generally without altering the biological activity of the resulting binding domain-immunoglobulin fusion protein molecule. For example, such substitutions are generally  
25 made by interchanging within the groups of polar residues, charged residues, hydrophobic residues, small residues, and the like. If necessary, such substitutions may be determined empirically merely by testing the resulting modified protein for the ability to bind to the appropriate cell surface receptors in *in vitro* biological assays, or to bind to appropriate antigens or desired target molecules.

The present invention further relates to nucleic acids which hybridize to constructs of the invention, including for example, binding domain-immunoglobulin fusion protein encoding polynucleotide sequences as provided herein, or their complements, as will be readily apparent to those familiar with the art, if there is at least about 70%, preferably at least about 80-85%, more preferably at least about 90%, and still more preferably at least about 95%, 96%, 97%, 98% or 99% identity between the sequences.

The present invention particularly relates to nucleic acids which hybridize under stringent conditions to, for example, the binding domain-immunoglobulin fusion encoding nucleic acids referred to herein. As used herein, to "hybridize" under conditions of a specified stringency is used to describe the stability of hybrids formed between two single-stranded nucleic acid molecules. Stringency of hybridization is typically expressed in conditions of ionic strength and temperature at which such hybrids are annealed and washed. The term "stringent conditions" refers to conditions that permit hybridization between polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent (*e.g.*, formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of organic solvents (*e.g.*, formamide), or raising the hybridization temperature. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in the presence of an organic solvent (*e.g.*, at least about 35% formamide, most preferably at least about 50% formamide). Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, for example, hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed, and are within the skill in the art. Other typical "high", "medium" and "low" stringency encompass

the following conditions or equivalent conditions thereto: high stringency: 0.1 x SSPE or SSC, 0.1% SDS, 65°C; medium stringency: 0.2 x SSPE or SSC, 0.1% SDS, 50°C; and low stringency: 1.0 x SSPE or SSC, 0.1% SDS, 50°C. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by altering the time, temperature and/or concentration of the solutions used for prehybridization, hybridization and wash steps, and suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation where a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

As used herein, preferred "stringent conditions" generally refer to hybridization that will occur only if there is at least about 90-95% and more preferably at least about 97% identity between the sequences. The nucleic acid constructs which hybridize to, for example, binding domain-immunoglobulin fusion encoding nucleic acids referred to herein, in preferred embodiments, encode polypeptides which retain substantially the same biological function or activity as, for example, the binding domain-immunoglobulin fusion polypeptides encoded by the cDNAs.

The nucleic acids of the present invention, also referred to herein as polynucleotides, may be in the form of RNA, for example, mRNA, or in the form of DNA, which DNA includes cDNA (also called "complementary DNA", which is a DNA molecule that is complementary to a specific messenger RNA), genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. A coding sequence which encodes a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide for use according to the invention may contain portions that are identical to the coding sequence known in the art or described herein for portions thereof, or may be a different coding sequence, which, as a result of the redundancy or degeneracy of the genetic code, encodes the same construct or portion thereof, including all or a portion of a binding domain-immunoglobulin fusion polypeptide.

The nucleic acids which encode constructs of the invention, for example, binding domain-immunoglobulin fusion polypeptides, for use according to the invention may include, but are not limited to: only the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide and additional coding sequence; the coding sequence for the construct, such as a binding domain-immunoglobulin fusion polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequences 5' and/or 3' of the coding sequence for the binding domain-immunoglobulin fusion polypeptide or a portion(s) thereof, which for example may further include but need not be limited to one or more regulatory nucleic acid sequences that may be a regulated or regulatable promoter, enhancer, other transcription regulatory sequence, repressor binding sequence, translation regulatory sequence or any other regulatory nucleic acid sequence. Thus, the term "nucleic acid encoding" or "polynucleotide encoding" a construct, for example, a binding domain-immunoglobulin fusion protein, encompasses a nucleic acid which includes only coding sequence for, for example, a binding domain-immunoglobulin fusion polypeptide as well as a nucleic acid which includes additional coding and/or non-coding sequence(s).

Nucleic acids and oligonucleotides for use as described herein can be synthesized by any method known to those of skill in this art (*see, e.g.*, WO 93/01286, U.S. Application Serial No. 07/723,454; U.S. Patent No. 5,218,088; U.S. Patent No. 5,175,269; U.S. Patent No. 5,109,124). Identification of various oligonucleotides and nucleic acid sequences also involves methods known in the art. For example, the desirable properties, lengths and other characteristics of oligonucleotides useful for cloning are well known. In certain embodiments, synthetic oligonucleotides and nucleic acid sequences may be designed that resist degradation by endogenous host cell nucleolytic enzymes by containing such linkages as: phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages that have proven useful in antisense applications. *See, e.g.*, Agrwal *et al.*, *Tetrahedron Lett.* **28**:3539-3542 (1987); Miller *et al.*, *J. Am. Chem. Soc.* **93**:6657-6665 (1971); Stec *et al.*, *Tetrahedron Lett.* **26**:2191-2194 (1985); Moody *et al.*, *Nucl. Acids Res.* **12**:4769-4782 (1989); Uznanski *et*

*al.*, *Nucl. Acids Res.* (1989); Letsinger *et al.*, *Tetrahedron* **40**:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* **54**:367-402 (1985); Eckstein, *Trends Biol. Sci.* **14**:97-100 (1989); Stein In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, Ed, Macmillan Press, London, pp. 97-117 (1989); Jager *et al.*, *Biochemistry* **27**:7237-7246 (1988).

5                   In one embodiment, the present invention provides truncated components (*e.g.*, binding domain polypeptide, hinge region polypeptide, linker, *etc.*) for use in a construct of the invention, for example, a binding domain-immunoglobulin fusion protein. In another embodiment the invention provides nucleic acids encoding a construct of the invention, for example, a binding domain-immunoglobulin fusion protein having such  
10 truncated components. A truncated molecule may be any molecule that comprises less than a full length version of the molecule of interest. Truncated molecules provided by the present invention may include truncated biological polymers, and in preferred embodiments of the invention such truncated molecules may be truncated nucleic acid molecules or truncated polypeptides. Truncated nucleic acid molecules have less than the full length  
15 nucleotide sequence of a known or described nucleic acid molecule, where such a known or described nucleic acid molecule may be a naturally occurring, a synthetic, or a recombinant nucleic acid molecule, so long as one skilled in the art would regard it as a full length molecule. Thus, for example, truncated nucleic acid molecules that correspond to a gene sequence contain less than the full length gene where the gene comprises coding and non-  
20 coding sequences, promoters, enhancers and other regulatory sequences, flanking sequences and the like, and other functional and non-functional sequences that are recognized as part of the gene. In another example, truncated nucleic acid molecules that correspond to a mRNA sequence contain less than the full length mRNA transcript, which may include various translated and non-translated regions as well as other functional and non-functional  
25 sequences.

                  In other preferred embodiments, truncated molecules are polypeptides that comprise less than the full length amino acid sequence of a particular protein or polypeptide component. As used herein "deletion" has its common meaning as understood by those familiar with the art, and may refer to molecules that lack one or more portions of a  
30 sequence from either terminus or from a non-terminal region, relative to a corresponding full



length molecule, for example, as in the case of truncated molecules provided herein. Truncated molecules that are linear biological polymers such as nucleic acid molecules or polypeptides may have one or more of a deletion from either terminus of the molecule and/or one or more deletions from a non-terminal region of the molecule, where such deletions may be deletions of from about 1-1500 contiguous nucleotide or amino acid residues, preferably about 1-500 contiguous nucleotide or amino acid residues and more preferably about 1-300 contiguous nucleotide or amino acid residues, including deletions of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31-40, 41-50, 51-74, 75-100, 101-150, 151-200, 201-250 or 251-299 contiguous nucleotide or amino acid residues. In certain particularly preferred embodiments truncated nucleic acid molecules may have a deletion of about 270-330 contiguous nucleotides. In certain more preferred embodiments, truncated polypeptide molecules may have a deletion, for example, of about 80-140 contiguous amino acids.

The present invention further relates to variants of the herein referenced nucleic acids that encode fragments, analogs and/or derivatives of a construct of the invention, for example, a binding domain-immunoglobulin fusion polypeptide. The variants of the nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be naturally occurring allelic variants of one or more portions of the the nucleic acid sequences included therein, or non-naturally occurring variants of such sequences or portions or sequences, including sequences varied by molecular engineering using, for example, methods known in the art for varying sequence. As is known in the art, an allelic variant is an alternate form of a nucleic acid sequence which may have at least one of a substitution, a deletion or an addition of one or more nucleotides, any of which does not substantially or undesireably alter the function of the encoded binding domain-immunoglobulin fusion polypeptide.

Variants and derivatives of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, may be obtained by mutations of nucleotide sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides or any portion thereof. Alterations of the native amino acid sequence may be accomplished by any of a number of conventional methods. Mutations can be introduced at particular loci,

for example, by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

5                   Alternatively, for example, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene wherein predetermined codons can be altered by substitution, deletion or insertion. Exemplary methods of making such alterations are disclosed by Walder *et al.*, 1986 *Gene* **42**:133; Bauer *et al.*, 1985 *Gene* **37**:73; Craik, January 1985 *BioTechniques* 12-19; Smith *et al.*, January 1985 *Genetic Engineering: Principles and Methods BioTechniques* 12-19; Costa GL, *et al.*, "Site-directed mutagenesis using a rapid PCR-based method," 1996 *Methods Mol Biol.* **57**:239-48; Rashtchian A., "Novel methods for cloning and engineering genes using the polymerase chain reaction," 1995 *Curr Opin Biotechnol.* **6**(1):30-6; Sharon J, *et al.*, "Oligonucleotide-directed mutagenesis of antibody combining sites," 1993 *Int Rev Immunol.* **10**(2-3):113-27; Kunkel, 15   1985 *Proc. Natl. Acad. Sci. USA* **82**:488; Kunkel *et al.*, 1987 *Methods in Enzymol.* **154**:367; and, U.S. Patent Nos. 4,518,584 and 4,737,462.

As an example, modification of DNA may be performed by site-directed mutagenesis of DNA encoding a protein combined with the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as 20   PCR splicing by overlap extension (SOE). Site-directed mutagenesis is typically effected using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well-known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used. See, *e.g.*, Veira *et al.*, 1987 *Meth. Enzymol.* **15**:3. In general, site-directed mutagenesis is performed by preparing a single- 25   stranded vector that encodes the protein of interest (*e.g.*, all or a component portion of a given binding domain-immunoglobulin fusion protein). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a 30   primer to produce a heteroduplex in which one strand encodes the altered sequence and the

other the original sequence. The heteroduplex is introduced into appropriate bacterial cells and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

5                   Equivalent DNA constructs that include code for additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed or desired for biological activity, for example, are also encompassed by the invention. For example, and as discussed above, sequences encoding Cys residues that are not desirable or essential for biological activity can be altered to cause the Cys residues to be  
10                   deleted or replaced with other amino acids, for example, thus preventing formation of incorrect or undesired intramolecular disulfide bridges upon synthesis or renaturation.

                  A "host cell" or "recombinant host cell" is a cell that contains a vector, *e.g.*, an expression vector, or a cell that has otherwise been manipulated by recombinant techniques to express a protein of interest. Host organisms include those organisms in  
15                   which recombinant production of constructs of the invention, for example, binding domain-immunoglobulin fusion products encoded by the recombinant constructs of the present invention may occur, such as bacteria (for example, *E. coli*), yeast (for example, *Saccharomyces cerevisiae* and *Pichia pastoris*), insect cells, and mammalian cells, including *in vitro* and *in vivo* expression. Host organisms thus may include organisms for the  
20                   construction, propagation, expression or other steps in the production of the compositions provided herein. Hosts include subjects in which immune responses take place, as described herein. Presently preferred host organisms for production of constructs of the invention that produce glycosylated proteins are mammalian cells or other cells systems that permit the expression and recovery of glycosylated proteins. Other cell lines include inbred murine  
25                   strains and murine cell lines, and human cells and cell lines.

                  A DNA construct encoding a desired construct of the invention, for example, a binding domain-immunoglobulin fusion protein, is introduced into a vector, for example, a plasmid, for expression in an appropriate host. In preferred embodiments, the host is a mammalian host, for example, a mammalian cell line. The sequence encoding the ligand or  
30                   nucleic acid binding domain is preferably codon-optimized for expression in the particular

host. Thus, for example, if a construct, for example, is a human binding domain-immunoglobulin fusion and is expressed in bacteria, the codons may be optimized for bacterial usage. For small coding regions, the gene can be synthesized as a single oligonucleotide. For larger proteins, splicing of multiple oligonucleotides, mutagenesis, or other techniques known to those in the art may be used. The sequences of nucleotides in plasmids or other vectors that are regulatory regions, such as promoters and operators, are operationally associated with one another for transcription. The sequence of nucleotides encoding a binding domain-immunoglobulin fusion protein may also include DNA encoding a secretion signal, whereby the resulting peptide is a precursor protein. The resulting processed protein may be recovered from the periplasmic space or the fermentation medium.

In preferred embodiments, the DNA plasmids may also include a transcription terminator sequence. As used herein, a "transcription terminator region" is a sequence that signals transcription termination. The entire transcription terminator may be obtained from a protein-encoding gene, which may be the same or different from the inserted binding domain-immunoglobulin fusion encoding gene or the source of the promoter. Transcription terminators are optional components of the expression systems herein, but are employed in preferred embodiments.

The plasmids or other vectors used herein include a promoter in operative association with the DNA encoding the protein or polypeptide of interest and are designed for expression of proteins in a suitable host as described above (*e.g.*, bacterial, murine, or human) depending upon the desired use of the plasmid (*e.g.*, administration of a vaccine containing binding domain-immunoglobulin fusion encoding sequences). Suitable promoters for expression of proteins and polypeptides herein are widely available and are well known in the art. Inducible promoters or constitutive promoters that are linked to regulatory regions are preferred. Such promoters include, for example, but are not limited to, the T7 phage promoter and other T7-like phage promoters, such as the T3, T5 and SP6 promoters, the *trp*, *lpp*, and *lac* promoters, such as the *lacUV5*, from *E. coli*; the P10 or polyhedrin gene promoter of baculovirus/insect cell expression systems (*see, e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784) and inducible promoters from other eukaryotic expression systems. For expression of the proteins such

promoters are inserted in a plasmid in operative linkage with a control region such as the lac operon.

Preferred promoter regions are those that are inducible and functional in mammalian cells, for example. Examples of suitable inducible promoters and promoter regions for bacterial expression include, but are not limited to: the *E. coli* lac operator responsive to isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG; see Nakamura *et al.*, 1979 *Cell* 18:1109-1117); the metallothionein promoter metal-regulatory-elements responsive to heavy-metal (*e.g.*, zinc) induction (see, *e.g.*, U.S. Patent No. 4,870,009); the phage T7lac promoter responsive to IPTG (see, *e.g.*, U.S. Patent No. 4,952,496; and Studier *et al.*, 1990 *Meth. Enzymol.* 185:60-89) and the TAC promoter. Depending on the expression host system to be used, plasmids may optionally include a selectable marker gene or genes that are functional in the host. Thus, for example, a selectable marker gene includes any gene that confers a phenotype on bacteria that allows transformed bacterial cells to be identified and selectively grown from among a vast majority of untransformed cells. Suitable selectable marker genes for bacterial hosts, for example, include the ampicillin resistance gene (Amp<sup>r</sup>), tetracycline resistance gene (Tc<sup>r</sup>) and the kanamycin resistance gene (Kan<sup>r</sup>). The kanamycin resistance gene is presently preferred for bacterial expression.

In various expression systems, plasmids or other vectors may also include DNA encoding a signal for secretion of the operably linked protein. Secretion signals suitable for use are widely available and are well known in the art. Prokaryotic and eukaryotic secretion signals functional in *E. coli* may be employed. Depending on the expression systems, presently preferred secretion signals may include, but are not limited to, those encoded by the following *E. coli* genes: ompA, ompT, ompF, ompC, beta-lactamase, and alkaline phosphatase, and the like (von Heijne, *J. Mol. Biol.* 184:99-105, 1985). In addition, the bacterial pelB gene secretion signal (Lei *et al.*, *J. Bacteriol.* 169:4379, 1987), the phoA secretion signal, and the cek2 functional in insect cell may be employed. The most preferred secretion signal for certain expression systems is the *E. coli* ompA secretion signal. Other prokaryotic and eukaryotic secretion signals known to those of skill in the art may also be employed (*see, e.g.*, von Heijne, *J. Mol. Biol.* 184:99-105, 1985). Using the methods described herein, one of skill in the art can substitute secretion

signals that are functional in either yeast, insect or mammalian cells to secrete proteins from those cells.

Preferred plasmids for transformation of *E. coli* cells include the pET expression vectors (*e.g.*, pET-11a, pET-12a-c, pET-15b; *see* U.S. Patent No. 4,952,496; available from Novagen, Madison, WI.). Other preferred plasmids include the pKK plasmids, particularly pKK 223-3, which contains the tac promoter (Brosius *et al.*, 1984 *Proc. Natl. Acad. Sci.* 81:6929; Ausubel *et al.*, *Current Protocols in Molecular Biology*; U.S. Patent Nos. 5,122,463, 5,173,403, 5,187,153, 5,204,254, 5,212,058, 5,212,286, 5,215,907, 5,220,013, 5,223,483, and 5,229,279). Plasmid pKK has been modified by replacement of the ampicillin resistance gene with a kanamycin resistance gene. (Available from Pharmacia; obtained from pUC4K, *see, e.g.*, Vieira *et al.* (1982 *Gene* 19:259-268; and U.S. Patent No. 4,719,179.) Baculovirus vectors, such as pBlueBac (also called pJVETL and derivatives thereof), particularly pBlueBac III (*see, e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may also be used for expression of the polypeptides in insect cells. Other plasmids include the pIN-IIIompA plasmids (*see* U.S. Patent No. 4,575,013; *see also* Duffaud *et al.*, *Meth. Enz.* 153:492-507, 1987), such as pIN-IIIompA2.

Preferably, if one or more DNA molecules is replicated in bacterial cells, the preferred host is *E. coli*. The preferred DNA molecule is such a system also includes a bacterial origin of replication, to ensure the maintenance of the DNA molecule from generation to generation of the bacteria. In this way, large quantities of the DNA molecule can be produced by replication in bacteria. In such expression systems, preferred bacterial origins of replication include, but are not limited to, the fl-ori and col E1 origins of replication. Preferred hosts for such systems contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter, such as the lacUV promoter (*see* U.S. Patent No. 4,952,496). Such hosts include, but are not limited to, lysogens *E. coli* strains HMS174(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3) and BL21(DE3). Strain BL21(DE3) is preferred. The pLys strains provide low levels of T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

The DNA molecules provided may also contain a gene coding for a repressor protein. The repressor protein is capable of repressing the transcription of a promoter that contains sequences of nucleotides to which the repressor protein binds. The promoter can be derepressed by altering the physiological conditions of the cell. For example, the alteration  
5 can be accomplished by adding to the growth medium a molecule that inhibits the ability to interact with the operator or with regulatory proteins or other regions of the DNA or by altering the temperature of the growth media. Preferred repressor proteins include, but are not limited to the *E. coli* lacI repressor responsive to IPTG induction, the temperature sensitive  $\lambda$  cI857 repressor, and the like. The *E. coli* lacI repressor is preferred.

10 In general, recombinant constructs of the subject invention will also contain elements necessary for transcription and translation. In particular, such elements are preferred where the recombinant expression construct containing nucleic acid sequences encoding binding domain-immunoglobulin fusion proteins is intended for expression in a host cell or organism. In certain embodiments of the present invention, cell type preferred  
15 or cell type specific expression of a cell binding domain-immunoglobulin fusion encoding gene may be achieved by placing the gene under regulation of a promoter. The choice of the promoter will depend upon the cell type to be transformed and the degree or type of control desired. Promoters can be constitutive or active and may further be cell type specific, tissue specific, individual cell specific, event specific, temporally specific or inducible. Cell-type  
20 specific promoters and event type specific promoters are preferred. Examples of constitutive or nonspecific promoters include the SV40 early promoter (U.S. Patent No. 5,118,627), the SV40 late promoter (U.S. Patent No. 5,118,627), CMV early gene promoter (U.S. Patent No. 5,168,062), and adenovirus promoter. In addition to viral promoters, cellular promoters are also amenable within the context of this invention. In particular,  
25 cellular promoters for the so-called housekeeping genes are useful. Viral promoters are preferred, because generally they are stronger promoters than cellular promoters. Promoter regions have been identified in the genes of many eukaryotes including higher eukaryotes, such that suitable promoters for use in a particular host can be readily selected by those skilled in the art.

Inducible promoters may also be used. These promoters include MMTV LTR (PCT WO 91/13160), inducible by dexamethasone; metallothionein promoter, inducible by heavy metals; and promoters with cAMP response elements, inducible by cAMP. By using an inducible promoter, the nucleic acid sequence encoding a binding domain-immunoglobulin fusion protein may be delivered to a cell by the subject invention expression construct and will remain quiescent until the addition of the inducer. This allows further control on the timing of production of the gene product.

Event-type specific promoters are active or up-regulated only upon the occurrence of an event, such as tumorigenicity or viral infection. The HIV LTR is a well known example of an event-specific promoter. The promoter is inactive unless the *tat* gene product is present, which occurs upon viral infection. Some event-type promoters are also tissue-specific.

Additionally, promoters that are coordinately regulated with a particular cellular gene may be used. For example, promoters of genes that are coordinately expressed may be used when expression of a particular binding construct of the invention, for example, a binding domain-immunoglobulin fusion protein-encoding gene is desired in concert with expression of one or more additional endogenous or exogenously introduced genes. This type of promoter is especially useful when one knows the pattern of gene expression relevant to induction of an immune response in a particular tissue of the immune system, so that specific immunocompetent cells within that tissue may be activated or otherwise recruited to participate in the immune response.

In addition to the promoter, repressor sequences, negative regulators, or tissue-specific silencers may be inserted to reduce non-specific expression of binding domain-immunoglobulin fusion protein encoding genes in certain situations, such as, for example, a host that is transiently immunocompromised as part of a therapeutic strategy. Multiple repressor elements may be inserted in the promoter region. Repression of transcription is independent on the orientation of repressor elements or distance from the promoter. One type of repressor sequence is an insulator sequence. Such sequences inhibit transcription (Dunaway *et al.*, 1997 *Mol Cell Biol* **17**: 182-9; Gdula *et al.*, 1996 *Proc Natl Acad Sci USA* **93**:9378-83, Chan *et al.*, 1996 *J Virol* **70**: 5312-28; Scott and Geyer, 1995



*EMBO J* 14:6258-67; Kalos and Fournier, 1995 *Mol Cell Biol* 15:198-207; Chung *et al.*, 1993 *Cell* 74: 505-14) and will silence undesired background transcription.

Repressor elements have also been identified in the promoter regions of the genes for type II (cartilage) collagen, choline acetyltransferase, albumin (Hu *et al.*, 1992 *J. Cell Growth Differ.* 3(9):577-588), phosphoglycerate kinase (PGK-2) (Misuno *et al.*, 1992 *Gene* 119(2):293-297), and in the 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase gene. (Lemaigre *et al.*, *Mol. Cell Biol.* 11(2):1099-1106). Furthermore, the negative regulatory element Tse-1 has been identified in a number of liver specific genes, and has been shown to block cAMP response element(CRE)-mediated induction of gene activation in hepatocytes. (Boshart *et al.*, 1990 *Cell* 61(5):905-916,).

In preferred embodiments, elements that increase the expression of the desired product are incorporated into the construct. Such elements include internal ribosome binding sites (IRES; Wang and Siddiqui, 1995 *Curr. Top. Microbiol. Immunol* 203:99; Ehrenfeld and Semler, 1995 *Curr. Top. Microbiol. Immunol.* 203:65; Rees *et al.*, 1996 *Biotechniques* 20:102; Sugimoto *et al.*, 1994 *Biotechnology* 12:694). IRES increase translation efficiency. As well, other sequences may enhance expression. For some genes, sequences especially at the 5' end inhibit transcription and/or translation. These sequences are usually palindromes that can form hairpin structures. Any such sequences in the nucleic acid to be delivered are generally deleted. Expression levels of the transcript or translated product are assayed to confirm or ascertain which sequences affect expression. Transcript levels may be assayed by any known method, including Northern blot hybridization, RNase probe protection and the like. Protein levels may be assayed by any known method, including ELISA, western blot, immunocytochemistry or other well known techniques.

Other elements may be incorporated into the constructs of the invention, for example, into binding domain-immunoglobulin fusion protein encoding constructs of the present invention. In preferred embodiments, the construct includes a transcription terminator sequence, including a polyadenylation sequence, splice donor and acceptor sites, and an enhancer. Other elements useful for expression and maintenance of the construct in mammalian cells or other eukaryotic cells may also be incorporated (*e.g.*, origin of replication). Because the constructs are conveniently produced in bacterial cells, elements

that are necessary for, or that enhance, propagation in bacteria are incorporated. Such elements include an origin of replication, a selectable marker and the like.

As provided herein, an additional level of controlling the expression of nucleic acids encoding constructs of the invention, for example, binding domain-immunoglobulin fusion proteins, delivered to cells for gene therapy, for example, may be provided by simultaneously delivering two or more differentially regulated nucleic acid constructs. The use of such a multiple nucleic acid construct approach may permit coordinated regulation of an immune response such as, for example, spatiotemporal coordination that depends on the cell type and/or presence of another expressed encoded component. Those familiar with the art will appreciate that multiple levels of regulated gene expression may be achieved in a similar manner by selection of suitable regulatory sequences, including but not limited to promoters, enhancers and other well known gene regulatory elements.

The present invention also relates to vectors, and to constructs prepared from known vectors that include nucleic acids of the present invention, and in particular to “recombinant expression constructs”, including any of various known constructs, including delivery constructs, useful for gene therapy, that include any nucleic acids encoding, for example, binding domain-immunoglobulin fusion proteins and polypeptides according to the invention as provided herein; to host cells which are genetically engineered with vectors and/or other constructs of the invention and to methods of administering expression or other constructs comprising nucleic acid sequences encoding, for example, binding domain-immunoglobulin fusion polypeptides and fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques.

Various constructs of the invention, including for example, binding domain-immunoglobulin fusion proteins, can be expressed in virtually any host cell, including *in vivo* host cells in the case of use for gene therapy, under the control of appropriate promoters, depending on the nature of the construct (*e.g.*, type of promoter, as described above), and on the nature of the desired host cell (*e.g.*, whether postmitotic terminally differentiated or actively dividing; *e.g.*, whether the expression construct occurs in host cell as an episome or is integrated into host cell genome).

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, by Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY, (1989); as noted herein, in particularly preferred embodiments of the invention, recombinant expression is conducted in

5 mammalian cells that have been transfected or transformed with the subject invention recombinant expression construct. See also, for example, Machida, CA., "Viral Vectors for Gene Therapy: Methods and Protocols"; Wolff, JA, "Gene Therapeutics: Methods and Applications of Direct Gene Transfer" (Birkhauser 1994); Stein, U and Walther, W (eds.P, "Gene Therapy of Cancer: Methods and Protocols" (Humana Press 2000); Robbins, PD

10 (ed.), "Gene Therapy Protocols" (Humana Press 1997); Morgan, JR (ed.), "Gene Therapy Protocols" (Humana Press 2002); Meager, A (ed.), "Gene Therapy Technologies, Applications and Regulations: From Laboratory to Clinic" (John Wiley & Sons Inc. 1999); MacHida, CA and Constant, JG, "Viral Vectors for Gene Therapy: Methods and Protocols" (Humana Press 2002); "New Methods Of Gene Therapy For Genetic Metabolic Diseases

15 NIH Guide," Volume 22, Number 35, October 1, 1993. See also recent U.S. patents relating to gene therapy, including vaccines, which include U.S. Pat. Nos. 6,384,210 ("Solvent for biopolymer synthesis, solvent microdroplets and methods of use"); 6,384,203 ("Family of immunoregulators designated leukocyte immunoglobulin-like receptors (LIR)"); 6,384,202 ("Cell-specific active compounds regulated by the cell cycle"); 6,384,018 ("Polynucleotide

20 tuberculosis vaccine"); 6,383,814 ("Cationic amphiphiles for intracellular delivery of therapeutic molecules"); 6,383,811 ("Polyampholytes for delivering polyions to a cell"); 6,383,795 ("Efficient purification of adenovirus"); 6,383,794 ("Methods of producing high titer recombinant adeno-associated virus"); 6,383,785 ("Self-enhancing, pharmacologically controllable expression systems"); 6,383,753 ("Yeast mammalian regulators of cell

25 proliferation"); 6,383,746 ("Functional promoter for CCR5"); 6,383,743 ("Method for serial analysis of gene expression"); 6,383,738 ("Herpes simplex virus ORF P is a repressor of viral protein synthesis"); 6,383,737 ("Human oxalyl-CoA Decarboxylase"); 6,383,733 ("Methods of screening for pharmacologically active compounds for the treatment of tumour diseases"); 6,383,522 ("Toxicity reduced composition containing an anti-neoplastic agent

30 and a shark cartilage extract"); 6,383,512 ("Vesicular complexes and methods of making and

using the same"); 6,383,481 ("Method for transplantation of hemopoietic stem cells"); 6,383,478 ("Polymeric encapsulation system promoting angiogenesis"); 6,383,138 ("Method for transdermal sampling of analytes"); 6,380,382 ("Gene encoding a protein having diagnostic, preventive, therapeutic, and other uses"); 6,380,371 ("Endoglycan: a novel protein having selectin ligand and chemokine presentation activity"); 6,380,369 ("Human DNA mismatch repair proteins"); 6,380,362 ("Polynucleotides, polypeptides expressed by the polynucleotides and methods for their use"); 6,380,170 ("Nucleic acid construct for the cell cycle regulated expression of structural genes"); 6,380,169 ("Metal complex containing oligonucleoside cleavage compounds and therapies"); 6,379,967 ("Herpesvirus saimiri as viral vector"); 6,379,966 ("Intravascular delivery of non-viral nucleic acid protease proteins, and uses thereof").

Typically, for example, expression constructs are derived from plasmid vectors. One preferred construct is a modified pNASS vector (Clontech, Palo Alto, CA), which has nucleic acid sequences encoding an ampicillin resistance gene, a polyadenylation signal and a T7 promoter site. Other suitable mammalian expression vectors are well known (see, e.g., Ausubel *et al.*, 1995; Sambrook *et al.*, *supra*; see also, e.g., catalogues from Invitrogen, San Diego, CA; Novagen, Madison, WI; Pharmacia, Piscataway, NJ; and others). Presently preferred constructs may be prepared that include a dihydrofolate reductase (DHFR) encoding sequence under suitable regulatory control, for promoting enhanced production levels of the binding domain-immunoglobulin fusion protein, which levels result from gene amplification following application of an appropriate selection agent (e.g., methotrexate).

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, as described above. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Thus, for example, the binding domain-immunoglobulin fusion protein encoding nucleic acids as provided herein may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing a binding domain-immunoglobulin fusion polypeptide in a host cell.

In certain preferred embodiments the constructs are included in formulations that are administered *in vivo*. Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies, or replication deficient retroviruses as described below. However, any other vector may be used for preparation of a recombinant expression construct, and in preferred embodiments such a vector will be replicable and viable in the host.

The appropriate DNA sequence(s) may be inserted into a vector, for example, by a variety of procedures. In general, a DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described, for example, in Ausubel *et al.* (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA); Sambrook *et al.* (1989 *Molecular Cloning*, Second Ed., Cold Spring Harbor Laboratory, Plainview, NY); Maniatis *et al.* (1982 *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, NY); Glover (Ed.) (1985 *DNA Cloning Vol. I and II*, IRL Press, Oxford, UK); Hames and Higgins (Eds.), (1985 *Nucleic Acid Hybridization*, IRL Press, Oxford, UK); and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequence(s) (*e.g.*, a constitutive promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include promoters of eukaryotic cells or their viruses, as described above. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred

recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding an binding domain-immunoglobulin fusion polypeptide is described herein.

Transcription of the DNA encoding proteins and polypeptides included within the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Gene therapy is the use of genetic material to treat disease. It comprises strategies to replace defective genes or add new genes to cells and/or tissues, and is being developed for application in the treatment of cancer, the correction of metabolic disorders and in the field of immunotherapy. Gene therapies of the invention include the use of various constructs of the invention, with or without a separate carrier or delivery vehicle or constructs, for treatment of the diseases, disorders, and/or conditions noted herein. Such constructs may also be used as vaccines for treatment or prevention of the diseases, disorders, and/or conditions noted herein. DNA vaccines, for example, make use of polynucleotides encoding immunogenic protein and nucleic acid determinants to stimulate the immune system against pathogens or tumor cells. Such strategies can stimulate either acquired or innate immunity or can involve the modification of immune function through cytokine expression. *In vivo* gene therapy involves the direct injection of genetic material into a patient or animal model of human disease. Vaccines and immune modulation are systemic therapies. With tissue-specific *in vivo* therapies, such as those that aim to treat cancer, localized gene delivery and/or expression/targeting systems are preferred. Diverse gene therapy vectors have been designed to target specific tissues, and procedures have been developed to physically target specific tissues, for example, using catheter-based technologies, all of which are contemplated herein. *Ex vivo* approaches to gene therapy are also contemplated herein and involve the removal, genetic modification, expansion and re-administration of a patient's own cells. Examples include bone marrow transplantation for

cancer treatment or the genetic modification of lymphoid progenitor cells. *Ex vivo* gene therapy is preferably applied to the treatment of cells that are easily accessible and can survive in culture during the gene transfer process (such as blood or skin cells).

Useful gene therapy vectors include adenoviral vectors, lentiviral vectors, Adeno-associated virus (AAV) vectors, Herpes Simplex Virus (Hsv) vectors, and retroviral vectors. Gene therapies may also be carried out using "naked DNA," liposome-based delivery, lipid-based delivery (including DNA attached to positively charged lipids), and electroporation.

As provided herein, in certain embodiments, including but not limited to gene therapy embodiments, the vector may be a viral vector such as, for example, a retroviral vector. Miller *et al.*, 1989 *BioTechniques* 7:980; Coffin and Varmus, 1996 *Retroviruses*, Cold Spring Harbor Laboratory Press, NY. For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

Retroviruses are RNA viruses which can replicate and integrate into the genome of a host cell via a DNA intermediate. This DNA intermediate, or provirus, may be stably integrated into the host cell DNA. According to certain embodiments of the present invention, an expression construct may comprise a retrovirus into which a foreign gene that encodes a foreign protein is incorporated in place of normal retroviral RNA. When retroviral RNA enters a host cell coincident with infection, the foreign gene is also introduced into the cell, and may then be integrated into host cell DNA as if it were part of the retroviral genome. Expression of this foreign gene within the host results in expression of the foreign protein.

Most retroviral vector systems which have been developed for gene therapy are based on murine retroviruses. Such retroviruses exist in two forms, as free viral particles referred to as virions, or as proviruses integrated into host cell DNA. The virion form of the virus contains the structural and enzymatic proteins of the retrovirus (including the enzyme reverse transcriptase), two RNA copies of the viral genome, and portions of the source cell

plasma membrane containing viral envelope glycoprotein. The retroviral genome is organized into four main regions: the Long Terminal Repeat (LTR), which contains *cis*-acting elements necessary for the initiation and termination of transcription and is situated both 5' and 3' of the coding genes, and the three coding genes *gag*, *pol*, and *env*. These  
5 three genes *gag*, *pol*, and *env* encode, respectively, internal viral structures, enzymatic proteins (such as integrase), and the envelope glycoprotein (designated gp70 and p15e) which confers infectivity and host range specificity of the virus, as well as the "R" peptide of undetermined function.

Separate packaging cell lines and vector producing cell lines have been  
10 developed because of safety concerns regarding the uses of retroviruses, including their use in expression constructs as provided by the present invention. Briefly, this methodology employs the use of two components, a retroviral vector and a packaging cell line (PCL). The retroviral vector contains long terminal repeats (LTRs), the foreign DNA to be transferred and a packaging sequence (*y*). This retroviral vector will not reproduce by itself  
15 because the genes which encode structural and envelope proteins are not included within the vector genome. The PCL contains genes encoding the *gag*, *pol*, and *env* proteins, but does not contain the packaging signal "*y*". Thus, a PCL can only form empty virion particles by itself. Within this general method, the retroviral vector is introduced into the PCL, thereby creating a vector-producing cell line (VCL). This VCL manufactures virion particles  
20 containing only the retroviral vector's (foreign) genome, and therefore has previously been considered to be a safe retrovirus vector for therapeutic use.

"Retroviral vector construct" refers to an assembly which is, within preferred embodiments of the invention, capable of directing the expression of a sequence(s) or gene(s) of interest, such as binding domain-immunoglobulin fusion encoding nucleic acid  
25 sequences. Briefly, the retroviral vector construct must include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis and a 3' LTR. A wide variety of heterologous sequences may be included within the vector construct, including for example, sequences which encode a protein (*e.g.*, cytotoxic protein, disease-associated antigen, immune accessory molecule, or replacement gene), or which are useful as a  
30 molecule itself (*e.g.*, as a ribozyme or antisense sequence).



Retroviral vector constructs of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses (see, *e.g.*, RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Such retroviruses may be readily  
5 obtained from depositories or collections such as the American Type Culture Collection ("ATCC"; Rockville, Maryland), or isolated from known sources using commonly available techniques. Any of the above retroviruses may be readily utilized in order to assemble or construct retroviral vector constructs, packaging cells, or producer cells of the present invention given the disclosure provided herein, and standard recombinant techniques (*e.g.*,  
10 Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Kunkle, 1985 *PNAS* 82:488).

Suitable promoters for use in viral vectors generally may include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, 1989 *Biotechniques* 7:980-990, or any other promoter  
15 (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and  $\beta$ -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either  
20 regulated promoters or promoters as described above.

As described above, the retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317,  $\psi$ -2,  $\psi$ -AM, PA12, T19-14X, VT-19-17-H2,  $\psi$ CRE,  $\psi$ CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in  
25 Miller, *Human Gene Therapy*, 1:5-14 (1990). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and  $\text{CaPO}_4$  precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptides or fusion proteins. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will  
5 express the nucleic acid sequence(s) encoding the binding domain-immunoglobulin fusion polypeptide or fusion protein. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, circulating peripheral blood mononuclear and polymorphonuclear cells including myelomonocytic cells, lymphocytes, myoblasts, tissue macrophages, dendritic cells, Kupffer  
10 cells, lymphoid and reticuloendothelia cells of the lymph nodes and spleen, keratinocytes, endothelial cells, and bronchial epithelial cells.

As another example of an embodiment of the invention in which a viral vector is used to prepare, for example, a recombinant binding domain-immunoglobulin fusion expression construct, in one preferred embodiment, host cells transduced by a  
15 recombinant viral construct directing the expression of binding domain-immunoglobulin fusion polypeptides or fusion proteins may produce viral particles containing expressed binding domain-immunoglobulin fusion polypeptides or fusion proteins that are derived from portions of a host cell membrane incorporated by the viral particles during viral budding.

20 In another aspect, the present invention relates to host cells containing the herein described nucleic acid constructs, such as, for example, recombinant binding domain-immunoglobulin fusion expression constructs. Host cells are genetically engineered (transduced, transformed or transfected) with the vectors and/or expression constructs of this invention which may be, for example, a cloning vector, a shuttle vector, or an expression  
25 construct. The vector or construct may be, for example, in the form of a plasmid, a viral particle, a phage, *etc.* The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as genes encoding binding domain-immunoglobulin fusion polypeptides or binding domain-immunoglobulin fusion fusion proteins. The culture

conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan.

The host cell for production or expression of a construct of the invention, for example, can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate host cells according to the present invention include, but need not be limited to, bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells, such as *Drosophila S2* and *Spodoptera Sf9*; animal cells, such as CHO, COS or 293 cells; adenoviruses; plant cells, or any suitable cell already adapted to *in vitro* propagation or so established *de novo*. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, 1981 *Cell* 23:175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences, for example as described herein regarding the preparation of binding domain-immunoglobulin fusion expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis *et al.*, 1986 *Basic Methods in Molecular Biology*).

The present invention constructs, for example, binding domain-immunoglobulin fusion proteins, or compositions comprising one or more polynucleotides encoding same as described herein (for example, to be administered under conditions and

for a time sufficient to permit expression of a binding domain-immunoglobulin fusion protein in a host cell *in vivo* or *in vitro*, for gene therapy, for example, among other things), may be formulated into pharmaceutical compositions for administration according to well known methodologies. Pharmaceutical compositions generally comprise one or more  
5 recombinant expression constructs, and/or expression products of such constructs, in combination with a pharmaceutically acceptable carrier, excipient or diluent. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. For nucleic acid-based formulations, or for formulations comprising expression products of the subject invention recombinant constructs, about 0.01 µg/kg to about 100 mg/kg body weight will be  
10 administered, for example, typically by the intradermal, subcutaneous, intramuscular or intravenous route, or by other routes. A preferred dosage, for example, is about 1 µg/kg to about 1 mg/kg, with about 5 µg/kg to about 200 µg/kg particularly preferred. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host. "Pharmaceutically acceptable carriers" for  
15 therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remingtons Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of  
20 *p*-hydroxybenzoic acid may be added as preservatives. *Id.* at 1449. In addition, antioxidants and suspending agents may be used. *Id.*

"Pharmaceutically acceptable salt" refers to salts of the compounds of the present invention derived from the combination of such compounds and an organic or inorganic acid (acid addition salts) or an organic or inorganic base (base addition salts). The  
25 compounds of the present invention may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present invention.

The pharmaceutical compositions that contain one or more nucleic acid constructs of the invention, for example, binding domain-immunoglobulin fusion protein encoding constructs (or their expressed products) may be in any form which allows for the  
30 composition to be administered to a patient. For example, the composition may be in the

form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral (*e.g.*, sublingually or buccally), sublingual, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques. The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of the invention in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present. Examples are sucrose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose and ethyl cellulose. Coloring and/or flavoring agents may be present. A coating shell may be employed.

The composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred compositions contain, in addition to one or more binding domain-immunoglobulin fusion construct or expressed product, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and

agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

5                   It may also be desirable to include other components in the preparation, such as delivery vehicles including but not limited to aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable microcapsules, and liposomes. Examples of immunostimulatory substances (adjuvants) for use in such vehicles include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopoly-saccharides (LPS),  
10                   glucan, IL-12, GM-CSF, gamma interferon and IL-15.

                  While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises  
15                   water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable  
20                   microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109. In this regard, it is preferable that the microsphere be larger than approximately 25 microns.

                  Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans,  
25                   chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (*e.g.*, sucrose) as diluents.

                  As described above, the subject invention includes compositions capable of  
30                   delivering nucleic acid molecules encoding binding domain-immunoglobulin fusion

proteins. Such compositions include recombinant viral vectors (e.g., retroviruses (see WO 90/07936, WO 91/02805, WO 93/25234, WO 93/25698, and WO 94/03622), adenovirus (see Berkner, 1988 *Biotechniques* 6:616-627; Li *et al.*, 1993 *Hum. Gene Ther.* 4:403-409; Vincent *et al.*, *Nat. Genet.* 5:130-134; and Kolls *et al.*, 1994 *Proc. Natl. Acad. Sci. USA* 91:215-219), pox virus (see U.S. Patent No. 4,769,330; U.S. Patent No. 5,017,487; and WO 89/01973)), recombinant expression construct nucleic acid molecules complexed to a polycationic molecule (see WO 93/03709), and nucleic acids associated with liposomes (see Wang *et al.*, 1987 *Proc. Natl. Acad. Sci. USA* 84:7851). In certain embodiments, the DNA may be linked to killed or inactivated adenovirus (see Curiel *et al.*, 1992 *Hum. Gene Ther.* 3:147-154; Cotton *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89:6094). Other suitable compositions include DNA-ligand (see Wu *et al.*, 1989 *J. Biol. Chem.* 264:16985-16987) and lipid-DNA combinations (see Felgner *et al.*, 1989 *Proc. Natl. Acad. Sci. USA* 84:7413-7417).

In addition to direct *in vivo* procedures, *ex vivo* procedures may be used in which cells are removed from a host, modified, and placed into the same or another host animal. It will be evident that one can utilize any of the compositions noted above for introduction of constructs of the invention, for example, binding domain-immunoglobulin fusion proteins or of binding domain-immunoglobulin fusion protein encoding nucleic acid molecules into tissue cells in an *ex vivo* context. Protocols for viral, physical and chemical methods of uptake are well known in the art.

Accordingly, the present invention is useful for treating a patient having a B cell disorder or a malignant condition, or for treating a cell culture derived from such a patient. As used herein, the term "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with cancer or a malignant condition, such as B cell lymphoma, or may be normal (*i.e.*, free of detectable disease and infection). A "cell culture" includes any preparation amenable to *ex vivo* treatment, for example a preparation containing immunocompetent cells or isolated cells of the immune system (including, but not limited to, T cells, macrophages, monocytes, B cells and dendritic cells). Such cells may be isolated by any of a variety of techniques well known to those of ordinary skill in the art (e.g., Ficoll-hypaque density centrifugation). The cells may (but need not) have been

isolated from a patient afflicted with a B cell disorder or a malignant condition, and may be reintroduced into a patient after treatment.

A liquid composition intended for either parenteral or oral administration should contain an amount of a construct of the invention, for example, a binding domain-immunoglobulin fusion protein encoding construct or expressed product, such that a suitable dosage will be obtained. Typically, this amount is at least 0.01 wt% of a binding domain-immunoglobulin fusion construct or expressed product in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Preferred oral compositions contain between about 4% and about 50% of binding domain-immunoglobulin fusion construct or expressed product(s). Preferred compositions and preparations are prepared so that, for example, a parenteral dosage unit contains between 0.01 to 1% by weight of active compound.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, beeswax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. Topical formulations may contain a concentration of a construct of the invention, for example, a binding domain-immunoglobulin fusion construct or expressed product, of from about 0.1 to about 10% w/v (weight per unit volume).

The composition may be intended for rectal administration, in the form, *e.g.*, of a suppository which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

In the methods of the invention, a construct of the invention, for example, a binding domain-immunoglobulin fusion encoding constructs or expressed product(s), may be administered through use of insert(s), bead(s), timed-release formulation(s), patch(es) or fast-release formulation(s).



Constructs of the invention, for example, antigen-binding constructs of the invention, may be administered or co-administered to an animal or patient in combination with, or at the same or about the same time, as other compounds. In one aspect, one or more constructs, including for example one or more antigen-binding constructs, are administered to an animal or patient in conjunction with one or more chemotherapeutic compounds such as alkylating agents, nucleoside analogues, and the like. The administration or co-administration of one or more constructs, including one or more antigen-binding constructs, of the invention and one or more chemotherapeutic agents can be used for the treatment of tumors or cancer in an animal or patient. Exemplary cancers include, but are not limited to, head and neck cancer, breast cancer, colorectal cancer, gastric cancer, hepatic cancer, bladder cancer, cervical cancer, endometrial cancer, lung cancer (non-small cell), ovarian cancer, pancreatic cancer, prostate cancer; choriocarcinoma (lung cancer); hairy cell leukemia, chronic lymphocytic leukemia, acute lymphocytic leukemia (breast & bladder), acute myelogenous leukemia, meningeal leukemia, chronic myelogenous leukemia, erythroleukemia. More commonly the cancers treated include non-Hodgkin's lymphoma (osteogenic sarcoma, adult soft tissue sarcoma), T-cell lymphoma, chronic lymphocytic leukaemia, slowly growing non-Hodgkin's lymphomas, Hodgkin's lymphoma and ovarian cancer.

Examples of an alkylating agents that can be co-administered with one or more constructs, including one or more antigen-binding constructs, of the invention include mechlorethamine, chlorambucil, ifosfamide, melphalan, busulfan, carmustine, lomustine, procarbazine, dacardazine, cisplatin, carboplatin, mitomycin C, cyclophosphamide, isosfamide, , hexamethylmelamine, thiotepa, , and dacarbazine, and analogues thereof. See for example U.S. Pat. No. 3,046,301 describing the synthesis of chlorambucil, U.S. Pat. No. 3,732,340 describing the synthesis of ifosfamide, U.S. Pat. No. 3,018,302 for the synthesis of cyclophosphamide, U.S. Pat. No. 3,032,584 describing the synthesis of melphalan, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed., McGraw-Hill, New York, NY, pp.536-544 (2001) for clinical aspects of cyclophosphamide, chlorambucil, melphalan, ifosfamide, procarbazine, hexamethylmelamine, cisplatin, and carboplatin. Examples of nucleoside analogues, include, but are not limited to, fludarabine pentostatin,

methotrexate, fluorouracil, fluorodeoxyuridine, CB3717, azacitidine, cytarabine, floxuridine, mercaptopurine, 6-thioguanine, , cladribine and analogues thereof. One example is the combination of constructs, including antigen-binding constructs, that bind CD20. This construct acts as a chemosensitising agent and works together with chemotherapeutic agents, such that less chemotherapeutic agents are necessary to achieve anti-tumor or anti-cancer effects. For example, U.S. Pat. No. 3,923,785 describing the synthesis of pentostatin, U.S. Pat. No. 4,080,325 describing the synthesis of methotrexate, U.S. Pat. No. 2,802,005 describing the synthesis of fluorouracil, and Braunwald et al., "Harrison's Principles of Internal Medicine," 15th Ed., McGraw-Hill, New York, NY, pp.536-544 (2001) for clinical aspects of methotrexate, 5-fluorouracil, cytosine arabinoside, 6-mercaptopurine, 6-thioguanine, and fludarabine phosphate.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered compounds that inhibit topoisomerase II or compounds that otherwise interact with nucleic acids in cells. Such compounds include, for example, doxorubicin, epirubicin, etoposide, teniposide, mitoxantrone, and analogues thereof. In one example, this combination is used in treatment to reduce tumor cell contamination of peripheral blood progenitor cells (PBSC) in conjunction with high-dose chemotherapy and autologous stem cell support (HDC-ASCT). See U.S. Patent 6,586,428 to Geroni *et al.*

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with therapeutic drugs. For example, Virulizin (Lorus Therapeutics), which is believed to stimulate the release of tumour necrosis factor, TNF-alpha, by tumour cells in vitro and stimulate activation of macrophage cells. This can be used in combination with one or more constructs, including one or more antigen-binding constructs, of the invention to increase cancer cell apoptosis and treat various types of cancers including Pancreatic Cancer, Malignant Melanoma, Kaposi's Sarcoma (KS), Lung Cancer, Breast Cancer, Uterine, Ovarian and Cervical Cancer. Another example is CpG 7909 (Coley Pharmaceutical Group), which is believed to activate NK cells and monocytes and enhance ADCC. This drug can be used in combination with cancer or tumor specific constructs, including antigen-

binding constructs, of the invention, such as an anti-CD20 construct, to treat non-Hodgkin's lymphoma and other cancers.

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with angiogenesis inhibitors to increase anti-tumor effects. Angiogenesis is the growth of new blood vessels. This process allows tumors to grow and metastasize. Inhibiting angiogenesis can help prevent metastasis, and stop the spread of tumors cells. Angiogenesis inhibitors include, but are not limited to, angiostatin, endostatin, thrombospondin, platelet factor 4, Cartilage-derived inhibitor (CDI), retinoids, Interleukin-12, tissue inhibitor of metalloproteinase 1, 2 and 3 (TIMP-1, TIMP-2, and TIMP-3) and proteins that block the angiogenesis signaling cascade, such as anti-VEGF (Vascular Endothelial Growth Factor) and IFN-alpha. Angiogenesis inhibitors can be administered or co-administered with tumor specific constructs, including antigen-binding constructs capable of mediating, for example, ADCC and/or complement fixation or chemotherapy-conjugated antigen-binding of the invention to combat various types of cancers, for example, solid tumor cancers such as lung and breast cancer.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with disease modifying anti-rheumatic agents (DMAR agents) for the treatment of rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes. In such treatment, the constructs, for example, antigen-binding constructs, of the invention are commonly administered in conjunction with compounds such as azathioprine, cyclosporin, gold, hydroxychloroquine, methotrexate, penicillamine, sulphasalazine, and the like.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with agents or compounds that counteract the biological effects of interleukin-1, including for example interleukin-1 inhibitors and interleukin-1 receptor antagonist. It is thought that interleukin-1 has a role in the generation of rheumatoid arthritis (RA), inflammation, and the destruction of joints. IL-1 inhibitors can also be used in conjunction with the constructs, including antigen-binding constructs, of the invention to treat arthritis, inflammatory bowel disease,

sepsis and septic shock, ischemic injury, reperfusion, ischemic brain injury such as cerebral palsy and multiple sclerosis. See U.S. Patent No. 6,159,460 to Thompson *et al.* In another aspect, for example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered to an animal or patient in  
5 conjunction with one or more glucocorticoids for example, methylprednisilone, dexamethasone, hydrocortisone, and the like. Glucocorticoids have been used to induce apoptosis and inhibit growth, independent of ADCC and CDC. These compounds can be combined with constructs, including antigen-binding constructs, of the invention capable of inducing apoptosis in cancer cells. In one example is the anti-CD20, and anti-CD40  
10 antigen-binding constructs, which can be used to induce apoptosis in B-cells, are combined with glucocorticoids to treat B-cell non-Hodgkin's lymphoma (NHL).

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with p38 inhibitors or antagonists. The p38 mitogen-activated protein kinase pathway is involved in a  
15 number of cellular processes instrumental to the development of rheumatoid arthritis. For example, the activation and infiltration of leukocytes as well as the production of inflammatory cytokines are p38-dependent processes.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention are administered or co-administered with compounds  
20 that promote the differentiation and proliferation of B-cells. Cytokines such as \ interleukin-4 (IL-4) and interleukin-6 (IL-6), in addition to other biological activities, have been shown to stimulate antibody synthesis and secretion by activated B lymphocytes. In a particular aspect of the invention, constructs, including antigen-binding constructs, that recognize and bind CD20 are co-administered with one or more of interleukin-4 (IL-4) and  
25 interleukin-6 (IL-6).

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with Interleukin-2 (IL-2). Interleukin 2 (IL-2) is a lymphokine that increases production of effector cells, such as CD4+ T-helper cells, CD8 cytotoxic cells, antibody producing B cells,  
30 natural killer cells (NK), and monocytes/macrophages. IL-2 helps produce T-cells, which in

turn secrete more of the IL-2 (an "autocrine loop"). IL-2 can be used to augment antibody-dependent cell-mediated cytotoxicity (ADCC) and immunotherapies associated with constructs of the invention. In one example, an anti-CD20 construct of the invention and IL-2 are used to treat patients with relapsed or refractory follicular non-Hodgkin's lymphoma.

5 In another example IL-2 is administered or co-administered with HIV immunotherapies to help with T cell recovery.

In another aspect one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with Interleukin-12 (IL-12). IL-12 is known to enhance cytolytic T-cell responses, promote the development of helper T cells, enhance the activity of natural killer (NK) cells, and induces the secretion of IFN- $\gamma$  in T and NK cells. IL-12 also increases many helper and effector cells that mediate apoptosis. In another aspect of the invention, one or more constructs, including one or more antigen-binding constructs, are administered or co-administered with IL-12 in the treatment of an animal or patient with a tumor or cancer. For example, a  
10 construct, including an antigen-binding construct, of the invention that binds CD20 combined with IL-2 for the treatment of a patient with B-cell non-Hodgkin's lymphoma (NHL).  
15

One or more constructs, including one or more antigen-binding constructs, of the invention can also be combined with immunomodulators to boost the efficacy of the antigen-binding constructs of the invention. Immunomodulators include, but are not limited to, Colony Stimulating Factors (CSF), Tumor necrosis Factors (TNF), and Interferons (IFN).  
20

CSFs can include granulocyte-macrophage CSF (GM-CSF), granulocyte-CSF (G-CSF), and macrophage CSF (M-CSF). GM-CSF is thought to regulate the development of neutrophils, macrophages, monocytes and eosinophils. G-CSF has been shown to induce neutrophil production, and M-CSF production. M-CSF has been shown to stimulate macrophages and monocytes. The use of CSFs to treat neutropenia in cancer patients has been long established. In one example, constructs, including antigen-binding constructs, of the invention can be combined with GM-CSF, G-CSF or combinations thereof  
25

in order to accelerate recovery from neutropenia in patients after bone marrow transplantation and chemotherapy. Neutrophils play a major role in fighting microbes such as bacterial, fungi and parasites. Patients with neutropenia are particularly susceptible to bacterial and wide spread fungal infections. In another example, a construct, including an antigen binding construct, of the invention can be combined with GM-CSF-treated neutrophils, monocytes and macrophages to increase activity against bacteria, fungi, etc, including the dreaded *Pneumocystis carinii*.

An example of an IFN is interferon alpha (IFN- $\alpha$ ). IFN- $\alpha$  is made naturally by some types of white blood cell as part of the immune response when the body reacts to cancers or viral infections. It has two main modes of attack, interfering with growth and proliferation of cancer cells and it boosting the production of killer T cells and other cells that attack cancer cells. Interferon is also thought to facilitate cancer cells to put out chemical signals that make them better targets for the immune system, and has been used in recent years for several different types of cancer, particularly kidney cancer, melanoma, multiple myeloma, and some types of leukemia. It is also used to treat viral infections such as hepatitis. Interferon-alpha2a, for example, enhances ADCC and can be combined with one or more constructs, including antigen-binding constructs, of the invention to increase the efficiency of ADCC activity associated with the construct. In another example, one or more constructs, including one or more antigen-binding constructs of the invention are administered or co-administered to an animal or patient with interferon-gamma (IFN- $\gamma$ ), which has been show to increase the number of anti-CD20 antigens on B cells and bone marrow plasma cells (BMPC). This is particularly useful for the treatment of patients with multiple myelomas, which have a reduced expression of CD20 in their B cells and bone marrow plasma cells (BMPC). Accordingly, the treatment of multiple myeloma patients with constructs, including antigen-binding constructs of the invention, in particular constructs that bind CD20, may be usefully co-administered in conjunction with IFN- $\gamma$

TNF is a class of natural chemicals with anticancer properties. One example of a TNF is TNF- alpha. TNF-alpha has also been shown to have synergistic effects with IFN-gamma and IL-12. In another example, TNF can be administered or co-administered

with one or more tumor specific constructs, including one or more antigen-binding constructs, of the invention, and include chemotherapy-conjugated antigen binding constructs of the invention, together with IFN-gamma, IL-12 or various combinations thereof. TNF is also known to be an inflammatory regulation molecule. TNF-alpha  
5 antibodies or antagonist(s) can be combined with anti-T cell constructs, including antigen-binding constructs, of the invention to treat patients with rheumatoid arthritis, psoriasis, ulcerative colitis, systemic lupus erythematosus (SLE), Crohn's disease, ankylosing spondylitis, and various inflammatory disease processes.

In another aspect, one or more constructs, including one or more antigen-  
10 binding constructs, of the invention can be administered or co-administered with another antibody or antigen-binding construct of the invention. One example is a construct, for example, an antigen-binding construct of the invention capable of binding CD20 combined with a construct capable of binding CD22, CD19 or combinations thereof. This combination is effective as a treatment for indolent and aggressive forms of B-cell lymphomas, and acute  
15 and chronic forms of lymphatic leukemias. See U.S. Patent 6,306,393 to Goldberg. In another example, constructs, including antigen-binding constructs, of the invention are co-administered with other constructs such as antigen-binding constructs of the invention that aid in mediating apoptosis. For example, a combination of one or more constructs, including one or more antigen-binding constructs of the invention capable of binding CD28,  
20 CD3, CD20 or a combination thereof. The combination of anti-CD28 and CD3 provides a method for prolonged proliferation of T-cells. See U.S. Patent No. 6,352,694 to June et al.. This prolonged T-cell proliferation increases the efficiency immune dependent cytotoxicity, particularly those associated with anti-CD20.

In another aspect, constructs, including antigen-binding constructs, of the  
25 invention can be administered or co-administered with one or more T-cell regulatory molecules. One example is a combination with interleukin-12 (IL-12). The IL-12 cytokine stimulates cell-mediated immunity, has angiostatic activity, and possesses significant anti-tumor effects in a variety of tumor models. IL-12 has also been shown to stimulate the production of interferon-gamma (IFN- $\gamma$ ). Accordingly, the treatment of multiple myeloma

patients with one or more constructs, including one or more antigen-binding constructs, of the invention, in particular those that bind CD20, is expected to be more efficacious when co-administered in conjunction with IL-12. In another example, one or more constructs, including one or more antigen-binding constructs, of the invention can be administered or co-administered with a binding-domain construct of the invention other protein capable of binding CTLA-4 to enhance the anti-tumor immune response, by inhibiting the downregulation of T-cell activation.

In another aspect, one or more constructs, including one or more antigen-binding constructs, of the invention can be combined with gene therapies. In one example, a chemotherapy-conjugated construct of the invention is administered or co-administered with the Bcl-2 antisense oligonucleotide. Bcl-2 is associated with tumor resistance to anti-cancer therapies, and its believed to blocking chemotherapy-induced cell death. In another example one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with an adenovirus for delivery of a "suicide gene." The adenovirus inserts the gene directly into the tumor cells, which makes these cells sensitive to an otherwise ineffective drug. Drug treatment then destroys the tumor cells, while leaving healthy cells untouched. However, once therapy is complete stray cancer cells that escaped therapy can reestablish and metastasize. Combining gene therapy with one or more constructs, including one or more antigen-binding constructs, will help kill stray cancer cells and minimize cancer reoccurrence.

A similar combination can be used with palliative (non-radical) operations to surgically remove tumors. In this example one or more constructs, including one or more antigen-binding constructs, of the invention can be administered before and after surgical extractions of tumors in order to increase the immune response and reduce the likelihood of reoccurrence by killing any cancer cells that were not removed during the surgery.

Another aspect combines a cancer or antigen vaccine and T-cell regulator molecules. For example, the binding portion, for example, an antigen-binding portion, of a construct can be specific for a cancer cell or antigen, or a protein fragment from a cancer cell or antigen. This can help mediate an immune response against a particular tumor or



antigen. Such constructs can be combined with T-cell regulators to increase the efficiency of the immune response.

In another example, one or more constructs, including one or more antigen-binding constructs, of the invention is administered or co-administered with retinoids. Retinoids include Vitamin A and its derivatives, which have the ability to stop cells from dividing and cause them to differentiate. Vitamin A is combined with an anti-cancer construct(s), including antigen-binding construct(s), of the invention to combat various forms of cancer.

The terms "binding construct" and "antigen-binding construct" as used herein may refer to, for example, engineered polypeptides, recombinant polypeptides, synthetic, semi-synthetic or other fusion proteins that are capable of binding a target, for example, an antigen. Antigen-binding constructs of the invention may be used in various applications, including those within the variety of uses to which antibodies or related immunoglobulin-type constructs may be put. Constructs, including antigen-binding constructs of the invention can be used in *in vivo* and *in vitro* experiments for therapeutic, diagnostic, research, and other purposes. Such uses include, for example, the following.

Constructs, including antigen-binding constructs of the invention may be used for immunohistochemistry applications. For example, they may be used for immunolocalization of a particular antigen or group of antigens in a tissue. Tissue can be fixed and incubated with antigen-binding constructs of interest. These constructs can then be localized using a secondary antibody or binding construct of the invention coupled to a label, for example, to a gold particle or an enzyme that gives a chemical reaction, like horseradish peroxidase or beta-galactosidase. A secondary antibody or binding construct is frequently made that is reactive against, for example, a portion of the primary binding construct. Thus, for example, if the primary binding construct has a human tail portion, the secondary antibody or binding construct could be, for example, a rabbit anti-mouse antibody or antigen-binding construct that has been linked to beta-galactosidase. Alternatively the antibody or binding construct of the invention can be purified and then conjugated to another molecule to produce a fluorescent antibody or binding construct.

Constructs, including antigen-binding constructs of the invention can also be used to detect the location of an antigen or antigens on the surface of cells or to detect the location of intracellular materials using, for example, Immunoelectron Microscopy. Electron dense materials such as ferritin or colloidal gold, for example, can be conjugated to an antigen-binding construct. Scanning electron microscopy can be used to detect the localization of the antigen/binding construct complex.

Constructs, including antigen-binding constructs of the invention may also be used to quantitate the presence of an antigen or antigens using one of a variety of immunoassay formats, for example, a radioimmunoassay (RIA) format or an enzyme-linked immunosorbent assay (ELISA) format. There are many variants of these approaches, but those are based on a similar idea. For example, if an antigen can be bound to a solid support or surface, or is in solution, it can be detected by reacting it with a specific antigen-binding construct of the invention. The presence or amount of the construct can then be detected or quantitated by reacting it with, for example, either a secondary antibody or a second antigen-binding construct of the invention by incorporating a label directly into the primary antibody. Alternatively, for example, an antigen-binding polypeptide of the invention can be bound to a solid surface and the antigen added. A second antibody or antigen-binding polypeptide(s) of the invention that recognizes a distinct epitope on the antigen can then be added and detected. This technique is commonly referred to as a "sandwich assay", which is frequently used to avoid problems of high background or non-specific reactions, among other reasons.

Because the binding constructs of the invention can have high affinity/affinities and/or selectivity/selectivities for a particular epitope or epitopes, they can also be used as affinity reagents, for example, in protein or antigen purification. In one example of such a process, antigen-binding constructs of the invention are immobilized on a suitable support, for example, Sephadex resin or filter paper. The immobilized construct is exposed to a sample containing, or suspected of containing, a target protein(s) or antigen(s). The support is rinsed with a suitable buffer that will remove unwanted materials. The support is washed with another buffer that will release the bound protein(s) or antigen(s).

Because particular binding constructs of the invention can bind to proteins or other antigens with high affinity and selectivity they can also be used as a criterion for the importance of a particular enzyme or other macromolecule in a particular reaction. If an antigen-binding construct of the invention can interfere with a reaction in a solution, this will indicate that the construct may be binding specifically to a protein or other antigenic material involved in that reaction.

Constructs, including antigen-binding constructs of the invention can also be used as receptor blockers or inhibitors or antagonists.

Constructs, including antigen-binding constructs of the invention can also be used in identifying and studying the function(s) of proteins. If an antigen-binding construct of the invention reacts with a specific protein, for example, that protein can subsequently be precipitated from solution, for example. Precipitation is typically performed by using a secondary antibody or antigen-binding construct of the invention that links primary complexes together. Alternatively, the complex can be removed by reacting the solution with either protein A or, for example, depending on the construct, an anti-Fc antibody, for example, which has been attached to beads, for example, so that can be easily removed from the solution.

Constructs, including antigen-binding constructs of the invention can also be used in conjunction with gel-shift experiments to identify specific nucleic acid-binding proteins such as DNA-binding proteins. For example, DNA-binding proteins can be assayed by their ability to bind with high affinity to a particular oligonucleotide. The mobility of an oligonucleotide associated with the protein is far different than the mobility of a free oligonucleotide and results in a gel migration pattern and signal that is commonly referred to as a gel shift. The addition of the construct to the binding assay can have either of two effects. If the construct binds to a region of a protein not involved in DNA binding it can result in a complex that has even a slower mobility and is detected as a greater shift in mobility (a super-shift). Alternatively, if the construct binds to a region of the protein involved in recognizing the DNA then it can disrupt the binding and eliminate the shift. In either case, the data from these experiments can serve as a criterion to identify a DNA-binding protein, for example.

It is also possible to use constructs, including antigen-binding constructs of the invention to detect a protein by western blotting after fractionation by SDS-PAGE, for example. Once fractionated proteins are transferred to a membrane such as a nitrocellulose sheet, they are exposed to a particular antigen-binding construct of the invention that specifically recognizes, or recognizes to a desired degree of selectivity, proteins immobilized to the blot. This allows particular proteins to be identified. This approach is particularly useful if the mobility of the protein changes during an experiment. For example, incorporation of a phosphate or a carbohydrate, or cleavage of the protein, results in a change in mobility that can be followed in straight forward manner by western analysis. With appropriate controls, this approach can be used to measure the abundance of a protein in response to experimental manipulations.

The combination of SDS gels and immunoprecipitation can also be extremely effective. If a particular protein can be immunoprecipitated in a solution, both supernatant and precipitated fractions can be separated on an SDS gel and studied using an antigen-binding constructs of the invention.

Sometimes a binding construct of the invention directed against one protein will also precipitate a second protein that interacts with the first protein. The second protein, as well as the first, can then be seen by staining the gel or by autoradiography. This relationship is frequently the first indication that a protein functions as part of a complex and it can also be used to demonstrate a physical interaction of two proteins that are hypothesized to interact on the basis of other evidence (*e.g.*, a two hybrid screen or a supressor mutation). This approach can be combined with western blotting analysis in several extremely effective ways.

Thus, for example, antigen-binding constructs of the invention can be used in a combination of immunoprecipitation and western analysis in the study, for example, of signal transduction and protein processing. For example, an immunoprecipitated protein can be subsequently studied by western analysis using a different antibody or antigen-binding construct of the invention that binds to the protein. The most useful of are those that are directed against particular structural determinants that may be present in a protein. Thus, an antibody or antigen-binding construct of the invention directed against a region of the

protein that undergoes proteolytic processing can be useful to follow proteolytic processing. Additionally, a construct of the invention or a mixture of antigen-binding constructs of the invention that recognize phosphorylated peptides (*e.g.*, anti PY (phosphorylated tyrosine) can be used to follow the extent of phosphorylation of a protein (using western analysis) after it is precipitated, or *visa versa*. Glycosylation reactions can also be followed by antigen-binding constructs of the invention directed against a carbohydrate epitope (or by lectins, *i.e.*, proteins that recognize carbohydrates). Likewise, some antigen-binding constructs of the invention can be made that specifically recognize a phosphorylated epitope, for example, that will recognize a tyrosine or a serine residue after phosphorylation, but will not bind (or detectably bind) the epitope in the absence of phosphate. This approach can be used to determine the phosphorylation state of a particular protein. For example, the phosphorylation of CREB (the cAMP response element binding protein) can be followed by an antibody that specifically recognizes an epitope in a way that is dependent on the phosphorylation of serine 133.

15                Constructs, including antigen-binding constructs of the invention can also be used to screen expression libraries to isolate candidate polynucleotides that express or present a particular epitope, or that have a particular affinity or expression characteristic.

              Constructs, including antigen-binding constructs of the invention that bind to a cell surface can also be used as a marker to quantitate the fraction of cells expressing that marker using flow cytometry. If different antigen-binding constructs of the invention / fluorescent dye combinations are used, for example, the fraction of cells expressing several antigens can be determined.

25                Constructs, including antigen-binding constructs of the invention that function like anti-idiotypic antibodies, *i.e.*, antibodies against the binding domain of another antibody, can be used in any of a number of methods in which it would be desirable or useful to mimic the structure of an antigen. Such uses include, for example, uses as cancer vaccines (including antigen-binding constructs of the invention that incorporate a molecular adjuvant), as probes for receptors, as receptor agonists, as receptor antagonists, as receptor blockers or inhibitors, and so on.

In another aspect, constructs, including antigen-binding constructs of the invention may be bispecific and thus capable of binding to two distinct epitopes, which may be present on the same or different cell types.

*In vivo* uses of constructs of the invention, including antigen-binding constructs, include therapy, alone or in combination with one or more other therapies, for various diseases including cancers as well as B-cell disorders including autoimmune diseases. In some cases the constructs of the invention are administered to a patient. In other cases, the construct may be coupled to another molecule by techniques known in the art, for example, a fluorescent molecule to aid in imaging a target, or a therapeutic drug and/or a toxin to aid in killing a target.

For example, a labeling molecule or atom can be conjugated or otherwise linked to the antigen-binding construct of the invention to aid in imaging or as a diagnostic agent. These include, but are not limited to enzymatic labels, radioisotopes or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds and bioluminescent compounds. Thus, binding constructs or antigen-binding constructs of the invention can be conjugated to a drug, which allows specific drug targeting and increased efficiency once the drug reaches the target. This facilitates drug therapy while reducing systemic toxicity and side effects. This allows use of drugs that would otherwise be unacceptable when administered systemically. Dosage will depend on the potency of the drug and the efficiency of the carrier construct. Other examples of *in vivo* uses include the use of binding constructs or antigen-binding constructs of the invention in which a toxin is chemically linked or conjugated to a polypeptide of the invention to form, for example, molecules that may be termed "immunoconjugates" or "immunotoxins." Typically, for example, such a toxin may include one or more radioisotopes (for example, Iodine-131, Yttrium-90, Rhenium-186, Copper-67, and/or Bismuth-212), natural toxins, chemotherapy agents, biological response modifiers, or any other substance that is capable of assisting in damaging or killing a target cell, inhibiting target cell replication, or is effective in disrupting a desired cellular function in a target cell.

The toxin portion of the immunotoxin can be derived from various sources. Toxins are commonly derived from plants or bacteria, but toxins of human origin or

synthetic toxins can be used as well, for example. Examples of toxins derived from bacteria or plants include, but are not limited to, abrin,  $\alpha$ -sarcin, diphtheria toxin, ricin, saporin, and *pseudomonas* exotoxin. Examples of mammalian enzymes include, but are not limited to, ribonucleases (RNase) and deoxyribonucleases. Numerous immunotoxins that may be used  
5 with one or more constructs of the invention have been described in the art. See, for example, U.S. Pat. No. 4,753,894 to Frankel *et al.*; U.S. Pat. No. 6,099,842 to Pastan *et al.*; Nevelle, *et al.*, 1982 *Immunol Rev.* **62**:75-91; Pastan *et al.*, 1992 *Ann Rev Biochem* **61**:331-354; Chaudary *et al.*, 1989 *Nature* **339**:394; and Batra *et al.*, 1991 *Mol. Cell. Biol.* **11**:2200. Modified toxins described herein and those described in the various publications are also  
10 within the scope of the instant invention.

Generally, the immunotoxins and other therapeutic agents of this invention are administered at a concentration that is therapeutically effective to treat or prevent a particular disease, disorder, or condition, such as for the treatment of tumors and malignancies, the treatment of autoimmune diseases, allergies and inflammation, *etc.* This  
15 effective dosage and mode of administration will depend on the animal or patient being treated, the disease or condition being treated, the strength of the immunoconjugates or immunotoxins and the efficiency of the conjugate. To accomplish this goal, the immunotoxins may be formulated using a variety of acceptable formulations and excipients known in the art. Typically, for example, the immunotoxins are administered by injection,  
20 either intravenously or intraperitoneally. Methods to accomplish this administration are known to those of ordinary skill in the art. In another aspect, the invention includes topically or orally administered compositions such as an aerosol or cream or patch that may be capable of transmission across mucous membranes.

Formulants may be added to an immunoconjugates or immunotoxins of the  
25 invention before administration to a patients being treated. A liquid formulation is most common, but other formulations are within the scope of the invention. The formulants may include for example oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking agents. Carbohydrates can include sugar or sugar alcohols such as mono, di, or polysaccharides, or water-soluble glucans. The saccharides or glucans  
30 can include for example fructose, dextrose, lactose, glucose, mannose, sorbose, xylose,

maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" may be defined as a C<sub>4</sub> to C<sub>8</sub> hydrocarbon having an -OH group and includes, for example, galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to the amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. In one aspect, the sugar or sugar alcohol concentration is between 0.5 w/v % and 15 w/v %, typically between 1.0 w/v % and 7.0 w/v %, more typically between 2.0 and 6.0 w/v %.

Exemplary amino acids include levorotary (L) forms of camitine, arginine, and betaine; however, other amino acids may be added. Commonly used polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, for example, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000, for example. A buffer can be used in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are more commonly utilized. The concentration can be, for example, from 0.01 to 0.3 molar. Higher or lower concentrations may be used.

Immunotoxins of the invention can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Exemplary polymers and methods to attach them to peptides are referenced in U.S. Pat. Nos. 4,766,106 to Katre *et al.*; 4,179,337 to Davis *et al.*; 4,495,285 to Shimizu *et al.*; and 4,609,546 to Hiratani.

The following Examples are offered by way of illustration and not by way of limitation.

## EXAMPLES

### EXAMPLE 1

#### CLONING OF THE 2H7 VARIABLE REGIONS AND CONSTRUCTION

#### AND SEQUENCING OF 2H7scFv-IG



This Example illustrates the cloning of cDNA molecules that encode the heavy chain and light chain variable regions of the monoclonal antibody 2H7. This Example also demonstrates the construction, sequencing, and expression of 2H7scFv-Ig.

Hybridoma cells expressing 2H7 monoclonal antibody that specifically  
5 bound to CD20 were provided by Ed Clark at the University of Washington, Seattle, WA. Prior to harvesting, hybridoma cells were kept in log phase growth for several days in RPMI 1640 media (Invitrogen/Life Technologies, Gaithersburg, MD) supplemented with glutamine, pyruvate, DMEM non-essential amino acids, and penicillin-streptomycin. Cells were pelleted by centrifugation from the culture medium, and  $2 \times 10^7$  cells were used to prepare  
10 RNA. RNA was isolated from the 2H7-producing hybridoma cells using the Pharmingen (San Diego, CA) total RNA isolation kit (Catalog # 45520K) according to the manufacturer's instructions accompanying the kit. One microgram (1  $\mu$ g) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 300 ng random primers were combined and denatured at 72 °C for 10 minutes prior to addition of enzyme.  
15 Superscript II reverse transcriptase (Life Technologies) was added to the RNA plus primer mixture in a total volume of 25  $\mu$ l in the presence of 5X second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 42°C for one hour.

The 2H7 cDNA generated in the randomly primed reverse transcriptase  
20 reaction and V region specific primers were used to amplify by PCR the variable regions for the light and heavy chain of the 2H7 antibody. The V region specific primers were designed using the published sequence (Genbank accession numbers M17954 for V<sub>L</sub> and M17953 for V<sub>H</sub>) as a guide. The two variable chains were designed with compatible end sequences so that an scFv could be assembled by ligation of the two V regions after amplification and  
25 restriction enzyme digestion.

A (gly<sub>4</sub>ser)<sub>3</sub> peptide linker to be inserted between the two V regions was incorporated by adding the extra nucleotides to the antisense primer for the V<sub>L</sub> of 2H7. A Sac I restriction site was also introduced at the junction between the two V regions. The sense primer used to amplify the 2H7 V<sub>L</sub>, that included a HindIII restriction site and the light  
30 chain leader peptide was 5'-gtc aag ctt gcc gcc atg gat ttg caa gtg cag att ttg cag c-3' (SEQ ID

NO: \_\_). The antisense primer was 5'-gtc gtc *gag ctc* cca cct cct cca gat cca cca ccg ccc gag cca ccg cca cct ttc agc tcc agc ttg gtc *cc*-3' (SEQ ID NO: \_\_). The reading frame of the V region is indicated as a bold, underlined codon. The Hind III and SacI sites are indicated by underlined italicized sequences.

5                   The V<sub>H</sub> domain was amplified without a leader peptide, but included a 5' SacI restriction site for fusion to the V<sub>L</sub> and a BclI restriction site at the 3' end for fusion to various tails, including the human IgG1 Fc domain and the truncated forms of CD40 ligand, CD154. The sense primer was 5'-gct gct *gag ctc tca* ggc tta tct aca gca agt ctg g-3' (SEQ ID NO: \_\_). The SacI site is indicated in italicized and underlined font, and the reading frame of the codon for the first amino acid of the V<sub>H</sub> domain is indicated in bold, underlined type.  
10                   The antisense primer was 5'-gtt gtc *tga tca gag* acg gtg acc gtg gtc cc-3' (SEQ ID NO: \_\_). The BclI site is indicated in italicized, underlined type, and the last serine of the V<sub>H</sub> domain sequence is indicated in bold, underlined type.

                  The scFv-Ig was assembled by inserting the 2H7 scFv HindIII-BclI fragment  
15                   into pUC19 containing the human IgG1 hinge, CH2, and CH3 regions, which was digested with restriction enzymes, HindIII and BclI. After ligation, the ligation products were transformed into DH5α bacteria. Positive clones were screened for the properly inserted fragments using the SacI site at the V<sub>L</sub>-V<sub>H</sub> junction of 2H7 as a diagnostic site. The 2H7scFv-Ig cDNA was subjected to cycle sequencing on a PE 9700 thermocycler using a  
20                   25-cycle program by denaturing at 96 °C for 10 seconds, annealing at 50 °C for 30 seconds, and extending at 72°C for 4 minutes. The sequencing primers were pUC forward and reverse primers and an internal primer that annealed to the CH2 domain human in the IgG constant region portion. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to  
25                   the manufacturer's instructions. Samples were subsequently purified using Centriscap columns (Catalog # CS-901, Princeton Separations, Adelphia, N.J.), the eluates dried in a Savant vacuum dryer, denatured in Template Suppression Reagent (PE-ABI), and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 6.0 (Informax, North Bethesda, MD) .  
30                   Figure 1 shows the cDNA and predicted amino acid sequence of the 2H7scFv-Ig construct.

## EXAMPLE 2

### EXPRESSION OF 2H7 SCFV-IG IN STABLE CHO CELL LINES

This Example illustrates expression of 2H7scFv-Ig in a eukaryotic cell line and characterization of the expressed 2H7scFv-Ig by SDS-PAGE and by functional assays, including ADCC and complement fixation.

The 2H7scFv-Ig HindIII-XbaI (~1.6 kb) fragment with correct sequence was inserted into the mammalian expression vector pD18, and DNA from positive clones was amplified using QIAGEN plasmid preparation kits (QIAGEN, Valencia, CA). The recombinant plasmid DNA (100 µg) was then linearized in a nonessential region by digestion with AscI, purified by phenol extraction, and resuspended in tissue culture media, Excell 302 (Catalog # 14312-79P, JRH Biosciences, Lenexa, KS). Cells for transfection, CHO DG44 cells, were kept in logarithmic growth, and 10<sup>7</sup> cells harvested for each transfection reaction. Linearized DNA was added to the CHO cells in a total volume of 0.8 ml for electroporation.

Stable production of the 2H7 scFv-Ig fusion protein (SEQ. ID NO:10) was achieved by electroporation of a selectable, amplifiable plasmid, pD18, containing the 2H7 scFv-Ig cDNA under the control of the CMV promoter, into Chinese Hamster Ovary (CHO) cells (all cell lines from American Type Culture Collection, Manassas, VA, unless otherwise noted). The 2H7 expression cassette was subcloned downstream of the CMV promoter into the vector multiple cloning site as a ~1.6 kb HindIII-XbaI fragment. The pD18 vector is a modified version of pcDNA3 encoding the DHFR selectable marker with an attenuated promoter to increase selection pressure for the plasmid. Plasmid DNA was prepared using Qiagen maxiprep kits, and purified plasmid was linearized at a unique AscI site prior to phenol extraction and ethanol precipitation. Salmon sperm DNA (Sigma-Aldrich, St. Louis, MO) was added as carrier DNA, and 100 µg each of plasmid and carrier DNA was used to transfect 10<sup>7</sup> CHO DG44 cells by electroporation. Cells were grown to logarithmic phase in Excell 302 media (JRH Biosciences) containing glutamine (4mM), pyruvate, recombinant insulin, penicillin-streptomycin, and 2X DMEM nonessential amino acids (all from Life Technologies, Gaithersburg, Maryland), hereafter referred to as "Excell 302 complete" media. Media for untransfected cells also contained HT (diluted from a 100X solution of

hypoxanthine and thymidine) (Invitrogen/Life Technologies). Media for transfections under selection contained varying levels of methotrexate (Sigma-Aldrich) as selective agent, ranging from 50 nM to 5  $\mu$ M. Electroporations were performed at 275 volts, 950  $\mu$ F. Transfected cells were allowed to recover overnight in non-selective media prior to selective plating in 96 well flat bottom plates (Costar) at varying serial dilutions ranging from 125 cells/well to 2000 cells/well. Culture media for cell cloning was Excell 302 complete, containing 100 nM methotrexate. Once clonal outgrowth was sufficient, serial dilutions of culture supernatants from master wells were screened for binding to CD20-CHO transfected cells. The clones with the highest production of the fusion protein were expanded into T25 and then T75 flasks to provide adequate numbers of cells for freezing and for scaling up production of the 2H7scFvIg. Production levels were further increased in cultures from three clones by progressive amplification in methotrexate containing culture media. At each successive passage of cells, the Excell 302 complete media contained an increased concentration of methotrexate, such that only the cells that amplified the DHFR plasmid could survive.

Supernatants were collected from CHO cells expressing the 2H7scFv-Ig, filtered through 0.2  $\mu$ m PES express filters (Nalgene, Rochester, NY) and were passed over a Protein A-agarose (IPA 300 crosslinked agarose) column (Repligen, Needham, MA). The column was washed with PBS, and then bound protein was eluted using 0.1 M citrate buffer, pH 3.0. Fractions were collected and eluted protein was neutralized using 1M Tris, pH 8.0, prior to dialysis overnight in PBS. Concentration of the purified 2H7scFv-Ig (SEQ ID NO: \_\_) was determined by absorption at 280 nm. An extinction coefficient of 1.77 was determined using the protein analysis tools in the Vector Nti Version 6.0 Software package (Informax, North Bethesda, MD). This program uses the amino acid composition data to calculate extinction coefficients.

Production levels of 2H7scFv-Ig by transfected, stable CHO cells were analyzed by flow cytometry. Purified 2H7scFv-Ig to CHO cells was allowed to bind to CHO cells that expressed CD20 (CD20 CHO) and analyzed by flow cytometry using a fluorescein-conjugated anti-human IgG second step reagent (Catalog Numbers H10101 and H10501, CalTag, Burlingame, CA). Figure 2 (top) shows a standard curve generated by

titration of 2H7scFv-Ig binding to CD20 CHO. At each concentration of 2H7scFv-Ig, the mean brightness of the fluorescein signal in linear units is shown. Supernatants collected from T flasks containing stable CHO cell clones expressing 2H7scFv-Ig were then allowed to bind to CD20 CHO and the binding was analyzed by flow cytometry. The fluorescein signal generated by 2H7scFv-Ig contained in the supernatants was measured and the 2H7scFv-Ig concentration in the supernatants was calculated from the standard curve (Figure 2, bottom).

Purified 2H7scFv-Ig (SEQ ID NO: \_\_) was analyzed by electrophoresis on SDS-Polyacrylamide gels. Samples of 2H7scFv-Ig, purified by independent Protein A Agarose column runs, were boiled in SDS sample buffer without reduction of disulfide bonds and applied to SDS 10% Tris-BIS gels (Catalog # NP0301, Novex, Carlsbad, CA). Twenty micrograms of each purified batch was loaded on the gels. The proteins were visualized after electrophoresis by Coomassie Blue staining (Pierce Gel Code Blue Stain Reagent, Catalog #24590, Pierce, Rockford, IL), and destaining in distilled water. Molecular weight markers were included on the same gel (Kaleidoscope Prestained Standards, Catalog # 161-0324, Bio-Rad, Hercules, CA). The results are presented in Figure 3. The numbers above the lanes designate independent purification batches. The molecular weights in kilodaltons of the size markers are indicated on the left side of the figure. Further experiments with alternative sample preparation conditions indicated that reduction of disulfide bonds by boiling the protein in SDS sample buffer containing DTT or 2-mercaptoethanol caused the 2H7scFv-Ig to aggregate.

Any number of other immunological parameters may be monitored using routine assays that are well known in the art. These may include, for example, antibody dependent cell-mediated cytotoxicity (ADCC) assays, secondary *in vitro* antibody responses, flow immunocytofluorimetric analysis of various peripheral blood or lymphoid mononuclear cell subpopulations using well established marker antigen systems, immunohistochemistry or other relevant assays. These and other assays may be found, for example, in Rose *et al.* (Eds.), *Manual of Clinical Laboratory Immunology*, 5<sup>th</sup> Ed., 1997 American Society of Microbiology, Washington, DC.

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos and Bjab. Rabbit complement (Pel-Freez, Rogers, AK) was used in the assay at a final dilution of 1/10. Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37 °C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 4A show that in the presence of rabbit complement, 2H7scFv-Ig lysed B cells expressing CD20.

The ability of 2H7scFv-Ig to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) was tested by measuring the release of <sup>51</sup>Cr from labeled Bjab cells in a 4-hour assay using a 100:1 ratio of PBMC to Bjab cells. The results shown in Figure 4B indicated that 2H7scFv-Ig can mediate antibody dependent cellular cytotoxicity (ADCC) because the release of <sup>51</sup>Cr was higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone.

### EXAMPLE 3

#### **EFFECT OF SIMULTANEOUS LIGATION OF CD20 AND CD40 ON GROWTH OF NORMAL B CELLS, AND ON CD95 EXPRESSION, AND INDUCTION OF APOPTOSIS**

This example illustrates the effect of cross-linking of CD20 and CD40 expressed on the cell surface on cell proliferation.

Dense resting B cells were isolated from human tonsil by a Percoll step gradient and T cells were removed by E-rosetting. Proliferation of resting, dense tonsillar B cells was measured by uptake of <sup>3</sup>[H]-thymidine during the last 12 hours of a 4-day experiment. Proliferation was measured in quadruplicate cultures with means and standard deviations as shown. Murine anti-human CD20 monoclonal antibody 1F5 (anti-CD20) was used alone or was cross-linked with anti-murine κ monoclonal antibody 187.1 (anti-CD20XL). CD40 activation was accomplished using soluble human CD154 fused with murine CD8 (CD154) (Hollenbaugh *et al.*, *EMBO J.* 11: 4212-21 (1992)), and CD40 cross-linking was accomplished using anti-murine CD8 monoclonal antibody 53-6 (CD154XL). This procedure allowed simultaneous cross-linking of CD20 and CD40 on the cell surface. The results are presented in Figure 5.

The effect of CD20 and CD40 cross-linking on Ramos cells, a B lymphoma cell line, was examined. Ramos cells were analyzed for CD95 (Fas) expression and percent apoptosis eighteen hours after treatment (no goat anti-mouse IgG (GAM)) and/or cross-linking (+GAM) using murine monoclonal antibodies that specifically bind CD20 (1F5) and CD40 (G28-5). Control cells were treated with a non-binding isotype control (64.1) specific for CD3.

Treated Ramos cells were harvested, incubated with FITC-anti-CD95, and analyzed by flow cytometry to determine the relative expression level of Fas on the cell surface after CD20 or CD40 cross-linking. Data is plotted as mean fluorescence of cells after treatment with the stimuli indicated (Figure 6A).

Treated Ramos cells from the same experiment were harvested and binding of annexin V was measured to indicate the percentage apoptosis in the treated cultures. Apoptosis was measured by binding of Annexin V 18 hours after cross-linking of CD20 and CD40 using 1F5 and G28-5 followed by cross-linking with GAM. Binding of Annexin V was measured using a FITC-Annexin V kit (Catalog # PN-IM2376, Immunotech, Marseille, France,). Annexin V binding is known to be an early event in progression of cells into apoptosis. Apoptosis, or programmed cell death, is a process characterized by a cascade of catabolic reactions leading to cell death by suicide. In the early phase of apoptosis, before cells change morphology and hydrolyze DNA, the integrity of the cell membrane is maintained but cells lose the asymmetry of their membrane phospholipids, exposing negatively charged phospholipids, such as phosphatidylserine, at the cell surface. Annexin V, a calcium and phospholipid binding protein, binds preferentially and with high affinity to phosphatidylserine. Results demonstrating the effect of cross-linking both CD20 and CD40 on expression of the FAS receptor (CD95) are presented in Figure 6B. The effect of cross-linking of both CD20 and CD40 on Annexin V binding to cells is shown in Figure 6B.

#### EXAMPLE 4

##### **CONSTRUCTION AND CHARACTERIZATION OF 2H7 ScFv-CD154 FUSION PROTEINS**

To construct a molecule capable of binding to both CD20 and CD40, cDNA encoding the 2H7 scFv was fused with cDNA encoding CD154, the CD40 ligand. The 2H7

scFv cDNA encoded on the HindIII-BclI fragment was removed from the 2H7 scFvIg construct, and inserted into a pD18 vector along with a BamHI-XbaI cDNA fragment encoding the extracellular domain of human CD154. The extracellular domain is encoded at the carboxy terminus of CD154, similar to other type II membrane proteins.

5                   The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). The primer sets included two different 5' or sense primers that created fusion junctions at two different positions within the extracellular domain of CD154. Two different fusion junctions were designed that resulted in a short or truncated form (form  
10 S4) including amino acids 108 (Glu)-261 (Leu) + (Glu), and a long or complete form (form L2) including amino acids 48 (Arg) -261 (Leu) + (Glu), of the extracellular domain of CD154, both constructed as BamHI-XbaI fragments. The sense primer which fuses the two different truncated extracellular domains to the 2H7scFv includes a BamHI site for cloning. The sense primer for the S4 form of the CD154 cDNA is designated SEQUENCE ID NO:  
15 11 or CD154BAM108 and encodes a 34 mer with the following sequence : 5'-gtt gtc gga tcc aga aaa cag ctt tga aat gca a-3' , while the antisense primer is designated SEQUENCE ID NO: 12 or CD154XBA and encodes a 44 mer with the following sequence: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'.

                  The oligonucleotide primers used in amplifying the long form (L2) of the  
20 CD154 extracellular domain encoding amino acids 48 (Arg)-261 (Leu) + (Glu), were as follows: The sense primer designated CD154 BAM48 (SEQUENCE ID NO:13) encoded a 35-mer with the following sequence: 5'-gtt gtc gga tcc aag aag gtt gga caa gat aga ag-3'. The antisense primer designated or CD154XBA (SEQUENCE ID NO:\_\_) encoded the 44-mer: 5'-gtt gtt tct aga tta tca ctc gag ttt gag taa gcc aaa gga cg-3'. Other PCR reaction conditions  
25 were identical to those used for amplifying the 2H7 scFv (see Example 1). PCR fragments were purified by PCR quick kits (QIAGEN, San Diego, CA), eluted in 30 µl ddH<sub>2</sub>O, and digested with BamHI and XbaI (Roche) restriction endonucleases in a 40 µl reaction volume at 37 °C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN), and ligated along with the 2H7  
30 HindIII-BclI fragment into the pD18 expression vector digested with HindIII+XbaI.



Ligation reactions were transformed into DH5-alpha chemically competent bacteria and plated onto LB plates containing 100 µg/ml ampicillin. Transformants were grown overnight at 37 °C, and isolated colonies used to inoculate 3 ml liquid cultures in Luria Broth containing 100 µg/ml ampicillin. Clones were screened after mini-plasmid preparations (QIAGEN) for insertion of both the 2H7 scFv and the CD154 extracellular domain fragments.

The 2H7scFv-CD154 construct cDNAs were subjected to cycle sequencing on a PE 9700 thermocycler using a 25-cycle program that included denaturing at 96 °C, 10 seconds, annealing at 50 °C for 5 seconds, and extension at 60°C, for 4 minutes. The sequencing primers used were pD18 forward (SEQ ID NO:\_\_: 5'-gtctatataagcagagctctggc-3') and pD18 reverse (SEQ ID NO:\_\_: 5'-cgaggctgatcagcgagctctagca-3') primers. In addition, an internal primer was used that had homology to the human CD154 sequence (SEQ ID NO:\_\_: 5'-ccgcaatttgaggattctgatcacc-3'). Sequencing reactions included primers at 3.2 pmol, approximately 200 ng DNA template, and 8 µl sequencing mix. Sequencing reactions were performed using the Big Dye Terminator Ready Sequencing Mix (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Samples were subsequently purified using Centrisep columns (Princeton Separations, Adelphia, NJ). The eluates were dried in a Savant speed-vacuum dryer, denatured in 20 µl template Suppression Reagent (ABI) at 95°C for 2 minutes, and analyzed on an ABI 310 Genetic Analyzer (PE-Applied Biosystems). The sequence was edited, translated, and analyzed using Vector Nti version 6.0 (Informax, North Bethesda, MD). The 2H7scFv-CD154 L2 cDNA sequence and predicted amino acid sequence is presented in Figure 7A, and 2H7scFv-CD154 S4 cDNA sequence and predicted amino acid sequence is presented in Figure 7B.

The binding activity of the 2H7 scFv-CD154 fusion proteins (SEQ. ID NO: \_\_ and \_\_) to CD20 and CD40 simultaneously was determined by flow cytometry. The assay used CHO cell targets that express CD20. After a 45-minute incubation of CD20 CHO cells with supernatants from cells transfected with the 2H7 scFv-CD154 expression plasmid, the CD20 CHO cells were washed twice and incubated with biotin-conjugated CD40-Ig fusion protein in PBS/2% FBS. After 45 min, cells were washed twice and

incubated with phycoerythrin (PE)-labeled streptavidin at 1:100 in PBS/2% FBS (Molecular Probes, Eugene OR). After an additional 30 min incubation, cells were washed 2X and were analyzed by flow cytometry. The results show that the 2H7 scFv-CD154 molecule was able to bind to CD20 on the cell surface and to capture biotin-conjugated CD40 from solution (Figure 8).

To determine the effect of the 2H7scFv-CD154 on growth and viability of B lymphoma and lymphoblastoid cell lines, cells were incubated with 2H7scFv-CD154 L2 (SEQ. ID NO: \_\_) for 12 hours and then examined for binding of Annexin V. Binding of Annexin V was measured using a FITC-Annexin V kit (Immunotech, Marseille, France, Catalog # PN-IM2376). B cell lines were incubated in 1 ml cultures with dilutions of concentrated, dialyzed supernatants from cells expressing secreted forms of the 2H7scFv-CD154 fusion proteins. The results are presented in Figure 9.

The growth rate of the Ramos B lymphoma cell line in the presence of 2H7scFv-CD154 was examined by uptake of <sup>3</sup>H-thymidine for the last 6 hours of a 24-hour culture. The effect of 2H7scFv-CD154 on cell proliferation is shown in Figure 10.

## EXAMPLE 5

### CONSTRUCTION AND CHARACTERIZATION OF CYTOXB ANTIBODY DERIVATIVES

CytoxB antibodies were derived from the 2H7 scFv-IgG polypeptide. The 2H7 scFv (see Example 1) was linked to the human IgG1 Fc domain via an altered hinge domain (see Figure 11). Cysteine residues in the hinge region were substituted with serine residues by site-directed mutagenesis and other methods known in the art. The mutant hinge was fused either to a wild-type Fc domain to create one construct, designated CytoxB-MHWTG1C, or was fused to a mutated Fc domain (CytoxB-MHMG1C) that had additional mutations introduced into the CH2 domain. Amino acid residues in CH2 that are implicated in effector function are illustrated in Figure 11. Mutations of one or more of these residues may reduce FcR binding and mediation of effector functions. In this example, the leucine residue 234 known in the art to be important to Fc receptor binding, was mutated in the 2H7 scFv fusion protein, CytoxB-[MG1H/MG1C]. In another construct, the human IgG1 hinge region was substituted with a portion of the human IgA hinge, which was fused to wild-type

human Fc domain (CytoxB-IgAHWTHG1C). (See Figure 11). This mutated hinge region allows expression of a mixture of monomeric and dimeric molecules that retain functional properties of the human IgG1 CH2 and CH3 domains. Synthetic, recombinant cDNA expression cassettes for these molecules were constructed and polypeptides were expressed in CHODG44 cells according to methods described in Example 2.

Purified fusion protein derivatives of CytoxB-scFvIg molecules were analyzed by SDS-PAGE according to the methods described in Example 2. Polyacrylamide gels were run under non-reducing and reducing conditions. Two different molecule weight marker sets, BioRad prestained markers, (BioRad, Hercules, CA) and Novex Multimark molecular weight markers were loaded onto each gel. The migration patterns of the different constructs and of Rituximab™ are presented in Figure 12.

The ability of the different derivatives of CytoxB-scFvIg molecules to mediate ADCC was measured using the Bjab B lymphoma cells as the target and freshly prepared human PBMCs as effector cells. (See Example 2). Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of Bjab cells per well remaining constant but the number of PBMCs were varied. Bjab cells were labeled for 2 hours with <sup>51</sup>Cr and aliquoted at a cell density of 5 x 10<sup>4</sup> cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 µg/ml to the various dilutions of PBMCs. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100 µl of culture supernatant was harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released. The results are presented in Figure 13.

Complement dependent cytotoxicity (CDC) activity of the CytoxB derivatives was also measured. Reactions were performed essentially as described in Example 2. The results are presented in Figure 14 as percent of dead cells to total cells for each concentration of fusion protein.

## EXAMPLE 6

### IN VIVO STUDIES IN MACAQUES

Initial *in vivo* studies with CytoxB derivatives have been performed in nonhuman primates. Figure 15 shows data characterizing the serum half-life of CytoxB in monkeys. Measurements were performed on serum samples obtained from two different macaques (J99231 and K99334) after doses of 6 mg/kg were administered to each monkey  
5 on the days indicated by arrows. For each sample, the level of 2H7scFvIg present was estimated by comparison to a standard curve generated by binding of purified CytoxB-(MHWTG1C)-Ig fusion protein to CD20 CHO cells (see Example 2). The data are tabulated in the bottom panel of the Figure 15.

The effect of CytoxB-(MHWTG1C)Ig fusion protein on levels of circulating  
10 CD40+ cells in macaques was investigated. Complete blood counts were performed at each of the days indicated in Figure 16. In addition, FACS (fluorescence activated cell sorter) assays were performed on peripheral blood lymphocytes using a CD40-specific fluorescein conjugated antibody to detect B cells among the cell population. The percentage of positive cells was then used to calculate the number of B cells in the original samples. The data are  
15 graphed as thousands of B cells per microliter of blood measured at the days indicated after injection (Figure 16).

#### EXAMPLE 7

##### **CONSTRUCTION AND EXPRESSION OF AN ANTI-CD19 scFv-IG FUSION PROTEIN**

An anti-CD19 scFv-Ig fusion protein was constructed, transfected into  
20 eukaryotic cells, and expressed according to methods presented in Examples 1, 2, and 5 and standard in the art. The variable heavy chain regions and variable light chain regions were cloned from RNA isolated from hybridoma cells producing antibody HD37, which specifically binds to CD19. Expression levels of a HD37scFv-IgAHWTG1C and a HD37scFv-IgMHWTG1C were measured and compared to a standard curve generated using  
25 purified HD37 scFvIg. The results are presented in Figure 17.

#### EXAMPLE 8

##### **CONSTRUCTION AND EXPRESSION OF AN ANTI-L6 scFv-IG FUSION PROTEIN**

An scFv-Ig fusion protein was constructed using variable regions derived from an anti-carcinoma monoclonal antibody, L6. The fusion protein was constructed,

transfected into eukaryotic cells, and expressed according to methods presented in Examples 1, 2, and 5 and standard in the art. Expression levels of L6 scFv-IgAH WCH2 CH3 and L6 scFv-(SSS-S)H WCH2 WCH3 were measured and compared to a standard curve generated using purified L6 scFvIg. The results are presented in Figure 18.

5

## EXAMPLE 9

### CHARACTERIZATION OF VARIOUS SCFV-IG FUSION PROTEINS

In addition to the scFv-Ig fusion protein already described, G28-1 (anti-CD37) scFv-Ig fusion proteins were prepared essentially as described in Examples 1 and 5. The variable regions of the heavy and light chains were cloned according to methods known  
10 in the art. ADCC activity of 2H7-MHWTG1C, 2H7-IgAHWTG1C, G28-1- MHWTG1C, G28-1 IgAHWTG1C, HD37- MHWTG1C, and HD37- IgAHWTG1C was determined according to methods described above (see Example 2). Results are presented in Figure 19. ADCC activity of L6scFv-IgAHWTG1C and L6scFv-IgMHWTG1C was measured using the 2981 human lung carcinoma cell line. The results are presented in Figure 20. The  
15 murine L6 monoclonal antibody is known not to exhibit ADCC activity.

The purified proteins were analyzed by SDS-PAGE under reducing and non-reducing conditions. Samples were prepared and gels run essentially as described in Examples 2 and 5. The results for the L6 and 2H7 scFv-Ig fusion proteins are presented in Figure 21 and the results for the G28-1 and HD37 scFv-Ig fusion proteins are presented in  
20 Figure 22.

## EXAMPLE 10

### CONSTRUCTION AND EXPRESSION OF ANTI-CD20 SCFV-LLAMA IG FUSION PROTEINS

This Example illustrates the cloning of llama IgG1, IgG2, and IgG3 constant region domains and the construction of immunoglobulin fusion proteins with each of the  
25 three constant regions and anti-CD20 scFv.

The constant regions of llama IgG1, IgG2, and IgG3 immunoglobulins were cloned and inserted into mammalian vector constructs containing an anti-CD20 single chain Fv, 2H7 scFv. Total RNA was isolated from peripheral blood mononuclear cells (PBMC) from llama blood (Triple J Farms, Bellingham, WA) by lysing the lymphocytes in TRIzol®

(Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. One microgram (1 µg) of total RNA was used as template to prepare cDNA by reverse transcription. The RNA and 200 ng random primers were combined and denatured at 72 °C for 10 minutes prior to addition of enzyme. Superscript II reverse transcriptase (Invitrogen Life Technologies) was added to the RNA plus primer mixture in a total volume of 25 µl in the presence of 5X second strand buffer and 0.1 M DTT provided with the enzyme. The reverse transcription reaction was allowed to proceed at 42 °C for one hour. The cDNA was amplified by PCR using sequence specific primers. The 5' primers were designed according to published sequences for the V<sub>HH</sub> and V<sub>H</sub> domains of camelids. The 3' primer, which was used to amplify all three isotypes, was designed using mammalian CH3 domain sequences as a guide. The following specific primers were used. The Bcl and XbaI sites are indicated by underlined italicized sequences.

5' primer for llama IgG1 constant region

LLG1-5'bgl: 5'-gtt gtt gat caa gaa cca cat gga gga tgc acg tg-3'

(SEQ ID NO:\_\_\_)

5' primer for llama IgG2 constant region

LLG2-5'bgl: 5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

(SEQ ID NO:\_\_\_)

5' primer for llama IgG3 constant region

LLG3-5'bgl: 5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'

(SEQ ID NO:\_\_\_)

3' primer for llama IgG1, IgG2, and IgG3 constant regions

LLG123-3'X: 5'-gtt gtt tct aga tta cta ttt acc cga aga ctg ggt gat gga-3'

(SEQ ID NO:\_\_\_)

PCR fragments of the expected size were cloned into TOPO® cloning vectors (Invitrogen Life Technologies) and then were sequenced. The sense sequencing primer, LLseqsense, had the sequence 5'-ctg aga tcg agt tca gct g-3' (SEQ ID NO:\_\_\_), and the antisense primer, LLseqAS, had the sequence 5'-cct cct ttg gct ttg tct c-3' (SEQ ID NO:\_\_\_). Sequencing was performed as described in Example 1. Figure 23 compares the amino acid sequence of the three isotype llama constant regions containing the hinge, CH2,

and CH3 domains with the amino acid sequence of human IgG1 hinge, CH2, and CH3 domains.

After verifying the sequence, the amplified PCR products were digested with restriction enzymes BclI and XbaI to create compatible restriction sites. The digested fragments were then gel-purified, and the DNA was eluted using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA). The 2H7scFv-Ig pD18 mammalian expression vector construct (see Example 2) was digested with BclI and XbaI to remove the human IgG hinge, CH2, and CH3 domains. The pD18 vector is a modified derivative of pCDNA3 that contains an attenuated DHFR gene, which serves as a selectable marker for mammalian expression (Hayden *et al.*, *Tissue Antigens* 48:242-54 (1996)). The purified llama IgG1, IgG2, and IgG3 constant region PCR products were ligated by T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN) into the double-digested 2H7 scFv-pD18 vector at room temperature overnight according to the manufacturer's instructions. After ligation, the ligation products were transformed into *E. coli* DH5 $\alpha$  bacteria (BD Biosciences, Palo Alto, CA) and plated according to standard molecular biology procedures and manufacturer's instructions. Isolated colonies were chosen to screen for transformants containing the correct inserts.

For expression of the encoded polypeptides, plasmid DNA from positive clones was transiently transfected into COS-7 cells using DEAE-dextran (Hayden *et al.*, *Ther Immunol.* 1:3-15 (1994)). COS-7 cells were seeded at approximately  $3 \times 10^6$  cells per 150 mm plate and grown overnight until the cells were about 75% confluent. Cells were then washed once with serum-free DMEM (Invitrogen Life Technologies, Grand Island, NY). Transfection supernatant (10 ml) containing 400  $\mu$ g/ml DEAE-dextran, 0.1 mM chloroquine, and 5  $\mu$ g/ml of the DNA constructs were added to the cells, which were then incubated at 37 °C for 3-4 hrs. After incubation, cells were pulsed with 10 ml of 10% dimethyl sulfoxide (DMSO) in 1x PBS at room temperature for 2 minutes. Cells were then placed back into fully supplemented DMEM/10% FBS (1% L-glutamine, 1% penicillin/streptomycin, 1% sodium pyruvate, 1% MEM essential amino acids) (Invitrogen Life Technologies). After 24 hours, the media was replaced with serum-free fully

supplemented DMEM (Invitrogen Life Technologies), and the cells were maintained up to 21 days with media changes every 3-4 days.

Ig-fusion proteins were purified by passing COS cell culture supernatants through Protein A Agarose (Repligen, Cambridge, MA) columns. After application of the culture supernatant, the Protein A columns were then washed with 1x PBS (Invitrogen Life Technologies). Bound Ig-fusion proteins were eluted with 0.1 M citric acid (pH 2.8), and the collected fractions were immediately neutralized with Tris base (pH 10.85). The fractions containing protein were identified by measuring the optical density ( $A_{280}$ ) and then were pooled, dialyzed against 1x PBS, (Invitrogen Life Technologies) and filtered through a 0.2  $\mu$ m filter.

The purified Ig-fusion proteins were analyzed by SDS-PAGE. Aliquots of 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and Rituxan® (Rituximab, anti-CD20 antibody, Genentech, Inc. and IDEC Pharmaceuticals Corp.) (provided by Dr. Oliver W. Press, Fred Hutchison Cancer Research Center, Seattle, WA) (5  $\mu$ g protein) were combined with 25  $\mu$ l 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples). Samples of each protein were also prepared in reducing sample buffer containing 5% 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO). Molecular weight markers (Invitrogen Life Technologies) were applied to the gels in non-reducing buffer only. The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies). After electrophoresis (approximately 1 hour), the gels were washed three times, five minutes each, with Distilled Water (Invitrogen Life Technologies) and then stained in 50 ml Bio-Safe Coomassie Stain (BioRad, Hercules, CA) overnight at room temperature. After a wash in Distilled Water, the gels were photographed. The migration pattern of each Ig-fusion protein is presented in Figure 24.

The ability of the 2H7 scFv-llama Ig fusion proteins to bind to cells expressing CD20 was demonstrated by flow cytometry. Serial dilutions starting at 25  $\mu$ g/ml of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, and 2H7 scFv-llama IgG3 were prepared and incubated with CD20-transfected (CD20+) CHO cells (from the laboratory of Dr. S. Skov, Institute of Medical Microbiology and Immunology, Copenhagen Denmark in 1% FBS 1x PBS media (Invitrogen Life Technologies) for one hour on ice. After the



incubation, the cells were then centrifuged and washed with 1% FBS in 1x PBS. To detect bound 2H7 scFv-llama Ig, the cells were incubated for one hour on ice with fluorescein-conjugated goat anti-camelid IgG (heavy and light chain) (1:100) (Triple J Farms). The cells were then centrifuged and resuspended in 1% FBS-1x PBS and analyzed using a  
5 Coulter Epics XL cell sorter (Beckman Coulter, Miami, FL). The data (percent of maximum brightness) are presented in Figure 25.

## EXAMPLE 11

### EFFECTOR FUNCTION OF ANTI-CD20 scFv-LLAMA Ig FUSION PROTEINS

10 This Example demonstrates the ability of anti-CD20 llama IgG1, IgG2, and IgG3 fusion proteins to mediate complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC).

The ability of the 2H7 scFv-llama IgG fusion proteins to kill CD20 positive cells in the presence of complement was tested using the BJAB human B cell line. Rabbit  
15 complement was obtained from 3-4 week old rabbits (Pel-Freez, Brown Deer, WI). BJAB cells ( $2 \times 10^6$  cells/ml) were combined with rabbit complement (final dilution 1:10) and purified 2H7 Ig fusion proteins. 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, and 2H7 scFv-human IgG1 wild type hinge-CH2-CH3) (Example 1) were added at 1:3 serial dilutions beginning at a concentration of 30  $\mu$ g/ml. After one hour at  
20 37 °C, cell viability was determined by counting live and dead cells by trypan blue exclusion (0.4%) (Invitrogen Life Technologies) using a hemacytometer (Bright-line, Horsham, PA). The percent killing was calculated by dividing the number of dead cells by the number of total cells (dead + live cells). The data presented in Figure 26 show that all Ig fusion proteins had CDC activity.

25 The ADCC activity of the 2H7 scFv-llama IgG fusion proteins was determined using BJAB cells as target cells and human or llama peripheral blood mononuclear cells (PBMC) as effector cells. BJAB cells were pre-incubated for approximately 2 hours with  $^{51}\text{Cr}$  (100  $\mu$ Ci) (Amersham Biosciences, Piscataway, NJ) in fully supplemented IMDM (Invitrogen Life Technologies) containing 15% FBS. The cells  
30 were mixed intermittently during the pre-incubation period. Fresh, resting human PBMC

were purified from whole blood using Lymphocyte Separation Media (LSM) (ICN Pharmaceuticals, New York, NY). PBMC were combined with labeled BJAB cells ( $5 \times 10^4$  cells per well of 96 well tissue culture plate) at ratios of 25:1, 50:1, and 100:1. To each combination was added 10  $\mu\text{g/ml}$  of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody. The mixtures were incubated for 6 hours at 37 °C. Supernatant from each reaction containing  $^{51}\text{Cr}$  released from lysed cells was collected onto a LumaPlate-96 filter plate (Packard, Meriden, CT), which was dried overnight. The amount of  $^{51}\text{Cr}$  was measured by a TopCount NXT plate reader (Packard). Figure 27 shows that the 2H7 scFv-llama IgG2 fusion protein was the most effective llama fusion protein in mediating ADCC. Each data point represents the average measurement of triplicate wells.

ADCC activity was affected by the source of effector cells. Llama PBMC were isolated from llama blood (Triple J Farms) using LSM. Llama effector cells were added at the same ratios to BJAB target cells as described for the ADCC assay using human effector cells. The cells were combined with 10  $\mu\text{g/ml}$  of purified 2H7 scFv-llama IgG1, 2H7 scFv-llama IgG2, 2H7 scFv-llama IgG3, Rituximab, or no anti-CD20 antibody. The results are presented in Figure 28.

## EXAMPLE 12

### CONSTRUCTION AND CHARACTERIZATION OF SCFV IG FUSION PROTEINS

#### EXPRESSED ON THE CELL SURFACE

This Example describes a retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors. The Example also demonstrates the effector function of these various scFv Ig fusion proteins expressed on the surface of target cells.

The heavy and light chain variable regions were cloned from murine monoclonal antibodies specific for various costimulatory receptors, and single chain Fv constructs were prepared essentially as described in Example 1. Antibodies included 2H7, anti-human CD20; 40.2.220, anti-human CD40; 2E12, anti-human CD28; 10A8, anti-human CD152 (anti-CTLA-4); and 500A2, anti-murine CD3. The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning

immunoglobulin genes and as described in Example 1. Single chain Fv constructs were prepared as described in Example 1 by inserting a nucleotide sequence encoding a (gly<sub>4</sub>ser)<sub>3</sub> peptide linker between the VL region nucleotide sequence of 40.2.220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs: \_\_, respectively) and the VH region nucleotide sequence of 40.2.220, 2E12, 10A8, and 500A2, respectively (SEQ ID NOs: \_\_, respectively). The polypeptide sequence for VL of 40.2.220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs: \_\_, respectively, and the polypeptide sequence for VH of 40.2.220, 2E12, 10A8, and 500A2 are set forth in SEQ ID NOs: \_\_, respectively. Each scFv polynucleotide (SEQ ID NOs: \_\_ for 40.2.220, 2E12, 10A8, and 500A2, respectively) was then fused to human IgG1 mutant hinge (CCC→SSS) and mutant CH2 (proline to serine mutation at residue 238 (238 numbering according to EU nomenclature, Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94; residue 251 according to Kabat *et al.*) and wild type CH3 domains according to the methods described in Example 5 and 11. Each scFv mutant IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO: \_\_), such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNAs encoding the scFv-IgG-CD80 fusion proteins (SEQ ID NOs: \_\_ for 40.2.220-, 2E12-, 10A8-, and 500A2-scFv-IgG-CD80, respectively) were inserted into the retroviral vector pLNCX (BD Biosciences Clontech, Palo Alto, CA) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral constructs were transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286). Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface.

CDC and ADCC assays were performed with the transfected Reh cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Reh cells expressing anti-human CD152 scFv-mutant IgG-CD80 (SEQ ID NO: \_\_); Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO: \_\_); Reh anti-human CD28 scFv- mutant IgG-CD80 (SEQ ID NO: \_\_); Reh anti-human CD40 scFv- mutant IgG-CD80 (SEQ ID NO: \_\_); Reh anti-human CD20 scFv- mutant IgG-CD80 (SEQ ID NO: \_\_)

were combined with human PBMC (see Example 11) and rabbit complement (10  $\mu$ g/ml) for one hour at 37 °C. Untransfected Reh cells were included as a control. Viability of the cells was determined by trypan blue exclusion, and the percent of killed cells was calculated (see Example 11). Figure 29 shows the effectiveness of the scFv-IgG-CD80 fusion proteins  
5 when expressed on the cell surface of tumor cells to mediate complement dependent cytotoxicity.

The same transfected Reh cells tested in the CDC assay plus Reh cells transfected with the polynucleotide construct that encodes anti-murine CD3-scFv-Ig-CD80 (SEQ ID NO:\_\_) were analyzed for ADCC activity (see Example 11). Untransfected and  
10 transfected Reh cells were pre-labeled with  $^{51}$ Cr (100  $\mu$ Ci) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the Reh target cells (5 x 10<sup>4</sup> cells per well of 96 well plate) at ratios of 5:1, 2.5:1, and 1.25:1. After five hours at 37 °C, culture supernatants were harvested and analyzed as described in Example 11. Percent specific killing was calculated according to the following equation: ((experiment  
15 release minus spontaneous release)/(maximum release minus spontaneous release)) x 100. The data are presented in Figure 30. Each data point represents the average of quadruplicate samples.

Using the same procedures described above, the same results with other binding domains were obtained using the following monoclonal antibodies monoclonal  
20 antibodies as sources of sFv: for CD20, 1F5 (Genbank AY 058907 and AY058906); for CD40, 2.36 and G28.5; for CD28, 9.3.

Cell surface expression of antibody binding domains is accomplished by fusing antibody scFvs to IgA hinge and constant regions and IgE hinge-acting region, *i.e.*, IgE CH2, and constant regions. Polynucleotides encoding an anti-4-1BB scFv, 5B9 (anti-  
25 human 4-1BB) scFv, and 2e12 (anti-human CD40) fused to IgAH IgA T4 (four terminal CH3 residues deleted) fused to the CD80 transmembrane and cytoplasmic domains and IgE Fc regions are shown in SEQ ID NOs:\_\_. The encoded polypeptides are shown in SEQ ID NOs:\_\_.

### EXAMPLE 13

## **CONSTRUCTION AND SEQUENCE OF HUMAN IG HINGE-CH2-CH3 MUTANTS AND 2H7**

### **VARIABLE REGION MUTANTS**

This Example describes construction of scFv fusion proteins containing mutant human IgG1 and IgA constant regions. This Example also describes construction of a 2H7 scFv mutant with a single point mutation in the variable heavy chain region. Mutations were introduced into variable and constant region domains according to methods described herein and known in the molecular biology arts. Figure 31 presents nomenclature for the Ig constant region constructs.

The human IgG1 hinge region of the 2H7 scFv human IgG1 hinge-CH2-CH3 fusion proteins was mutated to substitute cysteine residues that in a whole immunoglobulin are involved in forming disulfide bonds between two heavy chain molecules. One mutant, 2H7 scFv fused to a human IgG1 hinge region in which all three cysteine residues were mutated to serine residues (MTH (SSS)), was prepared as described in Example 5 (designated in Example 5 as CytoxB-MHWTG1C (includes wild type IgG1 CH2 and CH3 domains)) (now referred to as 2H7 scFv MTH (SSS) WTCH2CH3) and comprises the polynucleotide sequence SEQ ID NO:\_\_\_ encoding the polypeptide as set forth in SEQ ID NO\_\_\_. The polynucleotide sequence encoding this mutant (SEQ ID NO:\_\_\_) was used as a template to create mutant hinge regions in which the first two cysteine residues were substituted with serine residues (IgG MTH (SSC)). An oligonucleotide was designed to substitute the third serine residue with a cysteine and had the following sequence: 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g-3' (HuIgGMHncs3, SEQ ID NO:\_\_\_). A second mutant was prepared in which the mutant hinge had serine residues substituting the first and third cysteine residues (IgG MTH (SCS)). The sequence of the oligonucleotide to create this mutant was as follows: 5'-gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg-3' (HuIgGMHncs2, SEQ ID NO:\_\_\_). A third mutant was prepared with cysteine residues substituted at the second and third positions (IgG MTH (CSS)), also using the IgG MTH (SSS) mutant as template, and an oligonucleotide having the sequence, 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac-3' (HuIgGMHncs1, SEQ ID NO:\_\_\_).

The oligonucleotides introducing the mutations into the hinge region were combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-ggt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO:\_\_\_)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The

5 IgG MTH CSS and IgG MTH SCS mutant sequences were amplified for 25 cycles with a denaturation profile of 94 °C, annealing at 52 °C for 30 seconds, and extension at 72 °C for 30 seconds. The IgG MTH SSC mutant sequence was amplified under slightly different conditions: denaturation profile of 94 °C, annealing at 45 °C for 30 seconds, and extension at 72 °C for 45 seconds. The amplified polynucleotides were inserted into the TOPO®

10 cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10. The mutant hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CSS) WTCH2CH3 encoding

15 DNA (SEQ ID NO:\_\_\_); 2H7 scFv MTH (SCS) WTCH2CH3 encoding DNA (SEQ ID NO:\_\_\_); and 2H7 scFv MTH (SSC) WTCH2CH3 encoding DNA (SEQ ID NO:\_\_\_).

A mutation of leucine to serine at position 11 in the first framework region of the heavy chain variable region (numbering according to Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5<sup>th</sup> ed. Bethesda, MD: Public Health Service, National

20 Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:\_\_\_). The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11: 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO:\_\_\_). The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ

25 ID NO:\_\_\_) (XbaI site underlined and italicized). After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG MTH (SSS) WTCH2CH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ). The construct PSL1180-2H7 scFv-IgG MTH (SSS) WTCH2CH3 was digested with

30 Sac and XbaI to remove the wild type VH domain and the hinge and CH2 and CH3

domains. The PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and  
5 Example 10). The mutant is designated 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 (Figure 31). The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

Four constructs containing IgA constant region domains were prepared. One construct contained wild type IgA hinge fused to human IgG1 CH2 and CH3 (IgAH IgG  
10 WTCH2CH3) (Figure 31). Sequential PCR amplifications were performed to substitute the human IgG1 hinge of the 2H7 scFv construct with nucleotide sequences encoding the IgA hinge. The 5' oligonucleotide primer (huIgA/Gchim5) for the first PCR reaction had the sequence, 5'-cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg-3' (SEQ ID NO:\_\_). The primer (huIgAhg-5') for the second PCR reaction to add more IgA specific  
15 hinge sequence and add a BclI restriction enzyme site (*italicized and underlined*) had the sequence, 5'-gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc caa ct-3' (SEQ ID NO:\_\_). The 3' primer for both amplification steps was huIgG1-3' having the sequence, 5'-gtc tct aga cta tca ttt acc cgg aga cag-3' (SEQ ID NO:\_\_). The sequence of the PCR product was confirmed by TOPO ® cloning as described above. The gel-purified fragment  
20 was digested with BclI and XbaI and then inserted into the 2H7 scFv-pD18 vector that had been digested BclI and XbaI to remove all the IgG1 constant region domains. Ligation was performed as described in Example 10 to provide a mammalian expression vector comprising the nucleotide sequence (SEQ ID NO:\_\_) encoding a 2H7 scFv IgA hinge-IgG1 CH2-CH3 polypeptide (SEQ ID NO:\_\_).

25 A second pD18 mammalian expression vector was constructed that had a polynucleotide sequence (SEQ ID NO:\_\_) that encoded a 2H7 scFv fused to wild type IgA hinge, CH2, and CH3 domains (SEQ ID NO:\_\_). Human IgA constant regions sequences were obtained by using random primers to reverse transcribe total RNA isolated from human tonsil followed by PCR amplification of the cDNA using sequence specific primers,  
30 essentially as described in Example 10. Human IgA hinge-CH2-CH3 nucleotide sequence

(SEQ ID NO:\_\_\_\_) encoding the IgA-CH2-CH3 polypeptide (IgAH IgACH2CH3, Figure 31) (SEQ ID NO:\_\_\_\_) was amplified using the 5' oligonucleotide huIgAhg-5' (SEQ ID NO:(*same as above*\_\_\_\_)) and a 3' oligonucleotide huIgA3' having the sequence, 5'-gtt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac-3' (SEQ ID NO:\_\_\_\_). Secretion of a

5 2H7-IgA hinge-IgA CH2-CH3 polypeptide from transfected mammalian cells required co-expression of human J chain that covalently binds to two IgA CH3 domains via disulfide bonds. Total RNA was isolated from tonsil B cells and was reversed transcribed to generate cDNA as described above. PCR amplification of the nucleotide sequence encoding the J chain was performed with J chain specific primers. The 5' PCR primer, HUIJCH5nl, had the

10 sequence, 5'-gtt gtt aga tct caa gaa gat gaa agg att gtt ctt-3' (SEQ ID NO:\_\_\_\_), and sequence of the 3' primer, HUIJCH3, was 5'-gtt gtt tct aga tta gtc agg ata gca ggc atc tgg-3' (SEQ ID NO:\_\_\_\_). The cDNA was cloned into TOPO® for sequencing as described in Example 10. J chain encoding cDNA (SEQ ID NO:\_\_\_\_) was then inserted into pD18 and pCDNA3-Hygro (+) (Invitrogen Life Technology) vectors for co-transfection with 2H7 scFv IgA

15 hinge-CH2-CH3 constructs. The J chain has the predicted amino acid sequence set forth in SEQ ID NO:\_\_\_\_.

Secretion of an scFv IgA constant region construct in the absence of J chain was accomplished by engineering a truncated CH3 domain with a deletion of the four carboxy terminal amino acids (GTCY, SEQ ID NO:\_\_\_\_) (IgAH IgA-T4, Figure 31), which

20 include a cysteine residue that forms a disulfide bond with the J chain. The IgA hinge-CH2-CH3 nucleotide sequence containing the deletion in CH3 (SEQ ID NO:\_\_\_\_) was prepared using a 5' PCR primer (huIgAhg-5') having the sequence 5'-gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO:\_\_\_\_) (BclI site is underlined and italicized), and a 3' PCR primer (HUIGA3T1) having the sequence 5'-gtt gtt tct aga tta tca gtc cac ctc

25 cgc cat gac aac aga cac-3' (SEQ ID NO:\_\_\_\_). This mutated IgA constant region nucleotide sequence was inserted into a 2H7 scFv pD18 vector as described for the generation of the previous 2H7 scFv-Ig constructs (see Example 1 and this example) that comprises the polynucleotide sequence (SEQ ID NO:\_\_\_\_) encoding a 2H7 IgAH IgA-T4 polynucleotide (SEQ ID NO:\_\_\_\_).



A fourth construct was prepared that encoded a 2H7 scFv-IgA constant region fusion protein with a deletion of 14 additional amino acids, most of which are hydrophobic residues, from the carboxy terminus of IgA CH3. The 2H7 scFv-IgAH IgA-T4 encoding polynucleotide was used as template to engineer a deletion of the nucleotide sequence encoding PTHVNVSVVMAEVD (SEQ ID NO:\_\_\_). The 5' oligonucleotide primer had the sequence 5'-gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc tca act-3' (SEQ ID NO:\_\_\_) (BclI site shown as underlined and italicized). The 3' oligonucleotide sequence was 5'-gtt gtt tct aga tta tca tt acc cgc caa gcg gtc gat ggt ctt-3' (SEQ ID NO:\_\_\_). The deleted IgA CH3 region was amplified by using the above oligonucleotides to amplify the IgA constant region from RNA isolated from human tonsil such that the cDNA contained the deleted carboxyl terminal encoding region for the 18 amino acids. The IgAH IgA-T18 constant region was inserted into a 2H7 scFv pD18 vector that comprises the polynucleotide sequence (SEQ ID NO:\_\_\_) encoding a 2H7 IgAH IgA-T18 polynucleotide (SEQ ID NO:\_\_\_) as described above.

#### EXAMPLE 14

##### EFFECTOR FUNCTION OF CTLA-4 IGG FUSION PROTEINS

The Example compares the effector functions of CTLA-4 Ig fusion proteins in CDC and ADCC assays.

Two CTLA-4 IgG fusion proteins were constructed. One fusion protein comprises the extracellular domain of CTLA-4 fused to human IgG1 wild type hinge, CH2, and CH3 domains and is designated CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_\_). A pD18 mammalian expression vector comprising a polynucleotide sequence encoding CTLA-4 IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_\_) was prepared by fusing in frame the nucleotide sequence encoding the extracellular domain of CTLA-4 (SEQ ID NO:\_\_\_) (see U.S. Patent No. 5,844,095) to the nucleotide sequence encoding IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_\_) according to the methods described in Examples 1 and 10. The extracellular domain nucleotide sequence also comprises a BclI restriction enzyme site at the 3' end, and a leader peptide nucleotide sequence (SEQ ID NO:\_\_\_) that encodes an oncoM leader peptide (SEQ ID NO:\_\_\_). A second CTLA-4 IgG fusion protein,

designated CTLA-4 IgG MTH (SSS) MTCH2WTCH3, contained the extracellular domain of CTLA-4 (plus the oncoM leader peptide sequence) fused to a mutant IgG hinge in which all three cysteine residues were replaced with serine residues. The hinge region was fused to a mutant IgG1 CH2 domain that had a mutation at isotype position 238 (EU numbering, Ward *et al.*, *supra*, (position 251 using numbering according to Kabat *et al.*, *supra*; position 209 where numbering commences with first residue of IgG1 CH1; *i.e.*, PAPELLDGPS (SEQ ID NO:\_\_) of wild type IgG1 CH2 is modified to PAPELLDGSS (SEQ ID NO:\_\_)), which was fused to IgG1 wild type CH3 (U.S. Patent No. 5,844,095). The CTLA-4 IgG MTH (SSS) MTCH2WTCH3 polynucleotide comprises the nucleotide sequence in SEQ ID NO:\_\_ and the deduced amino acid sequence comprises the sequence provided in SEQ ID NO:\_\_. CTLA-4 fusion proteins were also prepared using CTLA-4 extracellular membrane encoding sequences without the leader peptide (SEQ ID NO:\_\_).

To measure CDC activity, purified CTLA-4 IgG WTH (CCC) WTCH2CH3 (2 µg/ml) or CTLA-4 IgG MTH (SSS) MTCH2WTCH3 (2 µg/ml) was added to Reh cells (see Example 12) and to Reh cells transfected with the costimulatory molecule CD80 such that CD80 was expressed on the cell surface (Reh CD80.10, obtained from Dr. E. Clark, University of Washington, Seattle, WA; see Doty *et al.*, 1998 *J. Immunol.* 161:2700; Doty *et al.*, 1996 *J. Immunol.* 157:3270), in the presence or absence of rabbit complement (10 µg/ml). Purified CTLA Ig fusion proteins were prepared from culture supernatants of transiently transfected COS cells according to methods described in Example 10. The assays were performed essentially as described in Example 11 and 12. The data presented in Figure 32 show that only CD80-transfected Reh cells were killed in the presence of complement and CTLA-4 IgG WTH (CCC) WTCH2CH3 fusion protein.

The purified CTLA-4 Ig fusion proteins were also tested in ADCC assays. Human PBMC, serving as effector cells, were added to Reh or Reh CD80.1 target cells at a ratio of 1.25:1, 2.5:1, 5.0:1, and 10:1. Cells were labeled and the assays performed essentially as described in Examples 11 and 12. The results are presented in Figure 33. Each data point represents the average of four independent culture wells at each effector:target cell ratio. The data show that only CTLA-4 IgG WTH (CCC) WTCH2CH3 mediated significant ADCC of Reh CD80.10 cells.

## EXAMPLE 15

### **EFFECTOR FUNCTION OF CTLA-4 IGA FUSION PROTEINS**

CTLA-4 IgA fusion proteins are prepared as described for the IgG fusion proteins (see Examples 1, 13, and 14). CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO:\_\_\_) is fused in open reading frame to nucleotides encoding IgAH IgACH2CH3 (SEQ ID NO:\_\_\_) to provide the nucleotide sequence (SEQ ID NO:\_\_\_) encoding a CTLA-4 IgAH IgACH2CH3 fusion protein (SEQ ID NO:\_\_\_). The fusion protein is transiently expressed in COS cells (see Example 10) or stably expressed in CHO cells (see Example 1). Secretion of the CTLA-4 IgAH IgACH2CH3 fusion protein requires co-transfection with a construct containing a polynucleotide sequence (SEQ ID NO:\_\_\_) that encodes human J chain (SEQ ID NO:\_\_\_). The CTLA-4 IgAH IgACH2CH3 fusion protein is isolated as described in Examples 10 and 14. To express a CTLA-4 IgA construct without the presence of J chain, a CTLA-4 IgAH IgA-T4 construct is prepared and transfected into mammalian cells. In a similar manner as described for the CTLA-4 extracellular fragment fused to wild type IgA hinge-CH2CH3, the CTLA-4 extracellular domain nucleotide sequence (SEQ ID NO:\_\_\_) is fused in open reading frame to a nucleotide sequence (SEQ ID NO:\_\_\_) encoding a IgAH IgA-T4 polypeptide (SEQ ID NO:\_\_\_) to provide a nucleotide sequence comprising SEQ ID NO:\_\_\_ encoding a CTLA-4 IgAH IgA-T4 polypeptide (SEQ ID NO:\_\_\_). Effector function of each construct is evaluated by CDC and ADCC as described in Example 14.

## EXAMPLE 16

### **BINDING OF ANTI-CD20 scFv HUMAN Ig FUSION PROTEINS**

#### **TO CHO CELLS EXPRESSING CD20**

This Example describes binding of 2H7 scFv Ig fusion proteins to CHO cells that express CD20. The analysis was performed by flow cytometry. Culture supernatants were collected from transiently transfected COS cells expressing 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_\_); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO:\_\_\_); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:\_\_\_); and 2H7 scFv VHSE11 WTH WTCH2CH3 (SEQ ID NO:\_\_\_), and two-fold serial dilutions were

prepared. Serial two-fold dilutions of purified 2H7 scFv IgG MTH (SSC) WTCH2CH3 (SEQ ID NO:\_\_\_) were prepared starting at a concentration of 5 µg/ml. The culture supernatants and purified fusion protein samples were incubated with (CD20+) CHO cells for one hour on ice. The cells were washed twice and then incubated with 1:100 FITC-conjugated goat anti-human IgG (CalTag) for 40 minutes. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 34.

### EXAMPLE 17

#### **IMMUNOBLOT ANALYSIS OF ANTI-CD20 scFv HUMAN IgG AND IGA FUSION PROTEINS**

10                    This Example describes immunoblot analysis of 2H7 scFv IgG and 2H7 scFv IgA fusion proteins that were immunoprecipitated from transfected cell culture supernatants.

                  COS cells were transiently transfected with plasmids comprising nucleotide sequences for 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO: ); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO: ); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO: ); 2H7 scFv IgA H IgG WTCH2CH3 (SEQ ID NO: ); and scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO: ) essentially according to the method described in Example 10. Cells were also transfected with vector only. After 48-72 hours at 37°C, cell culture supernatants were harvested and combined with protein A-agarose beads (Repligen) for one hour at 4 °C. The beads were centrifuged and washed several times in TNEN [20 mM Tris base, 100 mM NaCl, 1 mM EDTA, and 0.05% NP-40, pH 8.0). The immunoprecipitates were combined with 25 µl 2x NuPAGE® SDS Sample Buffer (Invitrogen Life Technologies) (non-reduced samples). The proteins were fractionated on NuPAGE® 10% Bis-Tris gels (Invitrogen Life Technologies). After electrophoresis (approximately 1 hour), the proteins were transferred from the gel onto a Immobilon P polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA) using a semi-dry blotter (Ellard Instrumentation, Monroe, WA). The PVDF membrane was blocked in PBS containing 5% nonfat milk and then probed with HRP-conjugated goat anti-human IgG (Fc specific) (CalTag). After washing the immunoblot several times in PBS, the blot was developed using ECL (Amersham Biosciences). The results are shown in Figure 35.

## EXAMPLE 18

### **BINDING OF ANTI-CD20 scFv HUMAN IGA FUSION PROTEINS TO CD20+ CHO CELLS**

This Example describes flow immunocytofluorimetry analysis of binding of 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO:\_\_\_) and 2H7 scFv IgAH IgAT4 (SEQ ID NO:\_\_\_) fusion proteins to (CD20+) CHO cells.

COS cells were transiently co-transfected as described in Example 10 with plasmid DNA comprising a polynucleotide sequence (SEQ ID NO:\_\_\_) encoding 2H7 scFv IgAH IgACH2CH3 polypeptide (SEQ ID NO:\_\_\_) and with a separate plasmid comprising a polynucleotide sequence (SEQ ID NO:\_\_\_) encoding a human J chain polypeptide (SEQ ID NO:\_\_\_). COS cells were also transfected with a polynucleotide sequence (SEQ ID NO:\_\_\_) encoding a 2H7 scFv IgA fusion protein that had a deletion of four amino acids at the carboxy terminus of CH3 (2H7 scFv IgAH IgA-T4, SEQ ID NO:\_\_\_). The transfections were performed as described in Example 10. Culture supernatants from transfected COS cells were combined with (CD20+) CHO cells (see Example 1) and incubated for one hour on ice. The cells were washed twice with PBS-2%FBS and then combined with FITC-conjugated goat anti-human IgA chain (CalTag) (1:100) for 40 minutes. The cells were again washed and then analyzed by flow cytometry using a Coulter Epics XL cell sorter. Figure 36 shows that co-transfection with J chain was not required for secretion of 2H7 scFv IgAH IgAT4, the 2H7 IgA fusion protein with the truncated CH3 carboxy end (SEQ ID NO:\_\_\_).

## EXAMPLE 19

### **EFFECTOR FUNCTION OF ANTI-CD20 scFv HUMAN IGA FUSION PROTEINS**

This Example illustrates ADCC activity of 2H7 IgG and IgA fusion proteins against cells that express CD20. BJAB cells were prelabeled with <sup>51</sup>Cr (100  $\mu$ Ci) (Amersham) for two hours at 37 °C. Effector cells were obtained from fresh, resting human whole blood, which was diluted in an equal volume of Alsever's solution to prevent coagulation. 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:\_\_\_); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO:\_\_\_); 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO:\_\_\_); and 2H7 scFv IgAH IgACH2CH3 (SEQ ID NO:\_\_\_) fusion proteins were

purified from transiently transfected COS cell supernatants (100-200 ml) by protein A chromatography as described in Example 10. COS cells transfected with the plasmid encoding 2H7 scFv IgAH IgACH2CH3 were co-transfected with a plasmid encoding human J chain as described in Example 18. Two-fold serial dilutions of the purified 2H7 Ig fusion proteins starting at 5  $\mu$ g/ml were added to the labeled BJAB cells ( $5 \times 10^4$  cells per well of 96 well tissue culture plate) in the presence of whole blood (100  $\mu$ l of whole blood diluted 1:1 in Alsever's solution, final dilution 1:4) and incubated for five hours at 37 °C. Culture supernatants were harvested and analyzed as described in Example 11. Percent specific killing was calculated according to the following equation: ((experiment release minus spontaneous release)/(maximum release minus spontaneous release)) x 100. The data are presented in Figure 37. Each data point represents the average of quadruplicate samples.

In a second ADCC assay, the number of labeled BJAB target cells was held constant in each sample, and whole blood was added at dilutions of 0.25, 0.125, and 0.0625. Purified 2H7 IgG and IgA fusion proteins were added at a concentration of 5  $\mu$ g/ml. The BJAB cells, whole blood, and fusion proteins were incubated, the supernatants harvested, and the percent specific killing was calculated as described above. Percent specific killing for each of the 2H7 fusion proteins is presented in Figure 38.

The ADCC activity of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (5  $\mu$ g/ml) and of purified 2H7 scFv IgAH IgACH2CH3 (5  $\mu$ g/ml) was compared in the presence of different effector cell populations. PBMC were isolated from whole blood as described in Examples 11 and 12. PBMC were combined with labeled BJAB target cells ( $5 \times 10^4$  per well of 96 well tissue culture plate) at ratios of 50:1, 25:1, and 12.5:1. The assay was performed and the data analyzed as described above. Figure 39A shows that only the 2H7 scFv IgG MTH (SSS) WTCH2CH3 fusion protein had ADCC activity when PBMC served as the effector cells. Figure 39B shows that both 2H7 scFv IgG MTH (SSS) WTCH2CH3 and 2H7 scFv IgAH IgACH2CH3 exhibit ADCC activity when whole blood was the source of effector cells (as illustrated in Figure 38).

## EXAMPLE 20

## **EXPRESSION LEVEL OF 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 FUSION**

### **PROTEIN**

This Example compares the expression level of 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:\_\_\_) with other 2H7 scFv IgG constructs that do not contain the mutation in the variable heavy chain domain. The mammalian expression vector pD18 comprising nucleotide sequences 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO: ); 2H7 scFv IgG MTH (CSS) WTCH2CH3 (SEQ ID NO: ); 2H7 scFv IgG MTH (SCS) WTCH2CH3 (SEQ ID NO: ); 2H7 scFv IgG WTH (CCC) WTCH2CH3 (SEQ ID NO: ); and 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 (see Examples 1 and 13) were transiently transfected into COS cells as described in Example 10. After 72 hours at 37 °C, culture supernatants were harvested, and 1 µl of each supernatant was combined with non-reducing sample buffer (see method described in Example 10). The culture supernatant samples and aliquots of purified 2H7 scFv IgG MTH (SSS) WTCH2CH3 (40 ng, 20 ng, 10 ng/ 5 ng, and 2.5 ng) were fractionated on 10% Bis-Tris (MOPS) NuPAGE® gels (Invitrogen Life Technologies). Multimark® protein standards (Invitrogen Life Technologies) were also separated on the gel. The proteins were transferred to a PDVF membrane and immunoblotted as described in Example 17. The immunoblot is presented in Figure 40. The amounts of the fusion proteins were quantified by densitometry analysis of the blots using the ScionImage for Windows software and comparison with the standard curve. The 2H7 scFv IgG WTH (CCC) WTCH2CH3 construct produced approximately 12 ng/ul or 12 micrograms/ml, the 2H7 scFv IgG MTH (CSS) WTCH2CH3 produced approximately 10 ng/ul or 10 micrograms/ml, the 2H7 scFv IgG MTH (SCS) WTCH2CH3 construct produced approximately 1 ng/ul or 1 microgram/ml, and the 2H7 scFv VH11Ser IgG MTH (SSS) WTCH2CH3 construct produced approximately 30 ng/ml or 30 micrograms/ml.

### **EXAMPLE 21**

#### **CONSTRUCTION OF A 2H7 scFv IgG FUSION PROTEIN WITH A MUTANT CH3 DOMAIN**

Amino acid mutations were introduced into the CH3 domain of a 2H7 IgG fusion protein. The pD18 vector comprising 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ

ID NO:\_\_) was digested with BclI and XbaI to remove the MTH WTCH2CH3 (SEQ ID NO:\_\_) fragment, which was then subcloned into pShuttle vector (BD Biosciences Clontech, Palo Alto, CA) that was double-digested with BclI and XbaI. Subcloning was performed in a kanamycin resistant vector because the ampicillin resistance gene has an XmnI site, which is required for this cloning procedure. Five constructs were prepared with the following substitutions: (1) a phenylalanine residue at position 405 (numbering according to Kabat *et al. supra*) was substituted with tyrosine using the oligonucleotide CH3Y405; (2) the phenylalanine position at 405 was substituted with an alanine residue using the oligonucleotide CH3A405; (3) the tyrosine residue at position 407 was substituted with an alanine using the oligonucleotide CH3A407; (4) both wild type amino acids at positions 405 and 407 were substituted with tyrosine and alanine, respectively using the oligonucleotide CH3Y405A407; and (5) both wild type amino acids at positions 405 and 407 were substituted with alanine using the oligonucleotide CH3A405A407. The oligonucleotides were the 3' primers for PCR amplification of a portion of the CH3 domain. The nucleotide sequences for each 3' oligonucleotide were as follows.

CH3Y405: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag gta gaa gga gcc-3' (SEQ ID NO:\_\_)

CH3A405: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct gta gag ggc gaa gga gcc-3' (SEQ ID NO:\_\_)

CH3A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gaa gaa gga gcc-3' (SEQ ID NO:\_\_)

CH3Y405A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag gta gaa gga gcc-3' (SEQ ID NO:\_\_)

CH3A405A407: 5'-gtt gtt gaa gac gtt ccc ctg ctg cca cct gct ctt gtc cac ggt gag ctt gct ggc gag ggc gaa gga gcc-3' (SEQ ID NO:\_\_)

The template was the mutant hinge MHWTCCH2CH3 human IgG1. The 5' PCR oligonucleotide primer was huIgGMHWC, [SEQ ID NO:\_\_]. The amplified products were TOPO® cloned and sequenced as described in Examples 1 and 10. DNA from the



clones with the correct sequence was digested with BclI and XmnI and transferred to pShuttle containing the MTH WTCH2CH3 sequence, which was also digested with the same restriction enzymes. The mutated IgG sequences were then removed by digestion with BclI and XbaI and inserted into a pD18 vector containing 2H7 scFv that was also digested with BclI and XbaI. The polynucleotide sequences for mutated the CH3 domains, MTCH3 Y405, MTCH3 A405, MTCH3 A407, MTCH3 Y405A407, and MTCH3 A405A407 are shown in SEQ ID NOs: \_\_\_\_, respectively, and the polypeptide sequences for each are shown in SEQ ID NOs: \_\_\_\_, respectively. The polynucleotide sequences for the 2H7 scFv MTH WTCH2 MTCH3 Y405, 2H7 scFv MTH WTCH2 MTCH3 A405, scFv MTH WTCH2 MTCH3 A407, scFv MTH WTCH2 MTCH3 Y405A407, and scFv MTH WTCH2 MTCH3 A405A407, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs: \_\_\_\_, respectively.

## EXAMPLE 22

### CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

A 2H7 scFv IgG fusion protein was constructed with the third cysteine residue in the IgG1 hinge region substituted with a serine residue. The template for introduction of the mutations was a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO: \_\_\_\_). The oligonucleotide introducing the mutations was a 5' PCR primer oligonucleotide HIgGMHcys3 having the sequence 5'-gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct-3'. The oligonucleotide introducing the mutation into the hinge region was combined with template and a 3' oligonucleotide containing an XbaI site (underlined and italicized) (5'-gtt gtt *tct aga* tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g-3' (SEQ ID NO: \_\_\_\_)) to amplify the mutant hinge-wild type (WT)-CH2-CH3 sequences by PCR. The IgG MTH CCS mutant sequence was amplified for 30 cycles with a denaturation profile of 94 °C, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds. The amplified polynucleotides were inserted into the TOPO® cloning vector (Invitrogen Life Technologies) and then were sequenced as described in Example 1 to confirm the presence of the mutation. pD18 vector containing 2H7 scFv was digested to remove the constant region sequences essentially as described in Example 10. The mutant

hinge-wild type CH2-CH3 regions were inserted in frame into the digested vector DNA to obtain vectors comprising 2H7 scFv MTH (CCS) WTCH2CH3 encoding DNA (SEQ ID NO:\_\_\_). The deduced polypeptide sequence is shown in SEQ ID NO:\_\_\_.

### EXAMPLE 23

#### 5                    CONSTRUCTION OF ANTI-CD20 IgE FUSION PROTEINS

A binding domain is fused to IgE constant region sequences such that the expressed polypeptide is capable of inducing an allergic response mechanism. The single chain Fv nucleotide sequence of 40.2.220 (SEQ ID NO:\_\_\_), an anti-CD40 antibody, is fused to IgE CH2-CH3-CH4 according to methods described for other scFv immunoglobulin  
10 constant region constructs (see Examples 1, 5, 10, and 13). To PCR amplify the IgE CH2-CH3-CH4 domains, a 5' oligonucleotide primer, hIgE5Bcl, having the sequence 5'-gtt gtt gat cac gtc tgc tcc agg gac ttc acc cc-3', and a 3' oligonucleotide primer, hIgE3stop, having the sequence 5'- gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g-3' are used.

The retroviral transfection system for ectopic surface expression of  
15 genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 40.2.220 scFv IgE-CD80 fusion protein. The 40.2.220 scFv IgE fusion polynucleotide sequence is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:\_\_\_), such that when the fusion protein is expressed in the transfected cell, CD80  
20 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the anti-CD40 scFv-IgE-CD80 fusion proteins (SEQ ID NO:\_\_\_) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The 40.2.220 scFv-Ig-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence.  
25 The retroviral constructs are transfected into a carcinoma cell line, and transfected cells are screened to select clones that are expressing the 40.2.220 scFv-Ig-CD80 fusion protein on the cell surface.

### EXAMPLE 24

#### CONSTRUCTION OF IGA-T4 MUTANTS THAT ARE EXPRESSED ON THE CELL SURFACE

The retroviral transfection system for ectopic surface expression of genetically engineered cell surface receptors composed of scFvs that bind costimulatory receptors described in Example 12 is used to construct a 2H7 scFv IgA hinge IgA-T4-CD80 fusion protein. The 2H7 scFv IgAH IgA-T4 fusion polynucleotide sequence (SEQ ID NO:\_\_) is fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:\_\_), such that when the fusion protein is expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the 2H7 scFv IgAH IgA-T4-CD80 fusion protein (SEQ ID NO:\_\_) is inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The 2H7 scFv IgAH IgA-T4-CD80 cDNA is inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence. The retroviral construct is transfected into Reh, an acute lymphocytic leukemia cell line (ATCC CRL-8286). Transfected cells are screened to select clones that are expressing 2H7 scFv-Ig fusion proteins on the cell surface.

## EXAMPLE 25

### **CHARACTERIZATION OF AN ANTI-4-1BB scFV Ig-CD80 FUSION PROTEIN EXPRESSED ON THE CELL SURFACE OF TUMOR CELLS AND GROWTH OF THE TUMOR CELLS *In Vivo***

This example describes construction of an anti-murine 4-1BB (CD137) scFv fusion protein that has an IgG wild type hinge and CH2 and CH3 domains that is fused to the CD80 transmembrane and cytoplasmic domains. The Example also illustrates the effect of the cell surface expression of the anti-4-1BB scFv IgG CD80 polypeptide when the transfected tumor cells are transplanted into mice.

The heavy and light chain variable regions of a rat anti-4-1BB (CD137) monoclonal antibody (1D8) were cloned, and a single chain Fv construct was prepared essentially as described in Example 1. The heavy chain and light chain variable regions of each antibody were cloned according to standard methods for cloning immunoglobulin genes and as described in Example 1. A single chain Fv construct was prepared as described in Example 1 by inserting a nucleotide sequence encoding a (gly<sub>4</sub>ser)<sub>3</sub> peptide linker between the VL region nucleotide sequence of 1D8 (SEQ ID NO:\_\_) and the VH region

nucleotide sequence of 1D8 (SEQ ID NO:\_\_\_). The polypeptide sequence for 1D8 VL is shown in SEQ ID NO:\_\_\_, and the polypeptide sequence for the VH domain is shown in SEQ ID NO:\_\_\_ . The scFv polynucleotide (SEQ ID NO:\_\_\_) was then fused to human IgG1 wild-type hinge-CH2-CH3 domains according to the methods described in Example 1. The scFv IgG1 fusion polynucleotide sequence was then fused in frame to sequences encoding the transmembrane domain and cytoplasmic tail of human CD80 (SEQ ID NO:\_\_\_) essentially as described in Example 12, such that when the fusion protein was expressed in the transfected cell, CD80 provided an anchor for surface expression of the scFv Ig fusion protein. cDNA encoding the scFv-IgG-CD80 fusion protein (SEQ ID NO:\_\_\_) was inserted into the retroviral vector pLNCX (BD Biosciences Clontech) according to standard molecular biology procedures and vendor instructions. The scFv-Ig-CD80 cDNA was inserted between the 5'LTR-neomycin resistance gene-CMV promoter sequences and the 3'LTR sequence.

The retroviral constructs were transfected into the metastatic M2 clone of K1735, a melanoma cell line, provided by Dr. I. Hellstrom, PNRI, Seattle, WA. Transfected cells were screened to select clones that were expressing scFv-Ig fusion proteins on the cell surface. To demonstrate that the 1D8 scFv IgG-CD80 construct was expressed on the cell surface of the tumor cells, the transfected cells were analyzed by flow immunocytofluorimetry. Transfected cells (K1735-1D8) were incubated for one hour on ice in phycoerythrin-conjugated F(ab')<sub>2</sub> goat anti-human IgG. The unbound conjugate was then removed by washing the cells and flow cytometry analysis was performed using a Coulter Epics XL cell sorter. Results are presented in Figure 41A.

The growth of K1735-1D8 transfected cells was examined *in vivo*. K1735-WT cells grew progressively when transplanted subcutaneously (s.c.) in naïve C3H mice. Although the same dose of K1735-1D8 cells initially formed tumors of an approximately 30 mm<sup>2</sup> surface area, the tumors started to regress around day 7 and had disappeared by day 20 as shown in Figure 41B. Tumor cells that were transfected with a similarly constructed vector encoding a non-binding scFv, a human anti-CD28 scFv construct, grew as well as tumor cells that had not been transfected. The presence of a foreign protein, that is, human IgG1 constant domains or rat variable regions, did not make transfected K1735-WT cells

immunogenic; the growth of the K1735-1D8 cells in C3H mice was identical to that of K1735-WT cells (untransfected).

To investigate the roles of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes and NK cells in the regression of K1735-1D8 tumors, naïve mice were injected intraperitoneally (i.p.) with monoclonal antibodies (monoclonal antibodies, typically 50 µg in a volume 0.1 ml) to remove CD8<sup>+</sup>, CD4<sup>+</sup> or both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, or were injected with anti-asialo-GM1 rabbit antibodies to remove NK cells. Twelve days later, when flow cytometry analysis of spleen cells from identically treated mice showed that the targeted T cell populations were depleted, K1735-1D8 cells were transplanted s.c to each T cell-depleted group. K1735-1D8 had similar growth kinetics in mice that had been injected with the anti-CD8 MAb or control rat IgG while removal of CD4<sup>+</sup> T cells resulted in the growth of K1735-1D8 with the same kinetics as K1735-WT. This failure to inhibit tumor growth after CD4<sup>+</sup> T cell removal was observed regardless of the presence or absence of CD8<sup>+</sup> T cells. K1735-1D8 grew in all NK-depleted mice, although more slowly than in the CD4-depleted group. The results are presented in Figure 41C.

## EXAMPLE 26

### THERAPEUTIC EFFECT OF TUMOR CELLS EXPRESSING ANTI-4-1BB scFv IgG-CD80

#### FUSION PROTEIN

This Example examines the ability of K1735-1D8 transfected tumor cells expressing an anti-CD137 scFv on the cell surface to generate a sufficient immune response in mice to mediate rejection of established, untransfected wild type tumors. C3H mice were transplanted with K1735-WT tumors (2x10<sup>6</sup> cells/animal) and grown for approximately six days. Experiments were performed using mice with established K1735-WT tumors of 30 mm<sup>2</sup> surface area. Mice were vaccinated by s.c. injection of K1735-1D8 or irradiated K1735-WT cells on the contralateral side. Identical injections were repeated at the time points indicated in Figure 42. One group of animals was given four weekly injections of K1735-1D8 cells. According to the same schedule, another group was given irradiated (12,000 rads) K1735-WT cells, and a third group was injected with PBS. The data are

plotted in Figure 42. The WT tumors grew progressively in all control mice and in all mice that received irradiated K1735-WT cells. In contrast, the tumors regressed in 4 of the 5 mice treated by immunization with K1735-1D8. The animals remained tumor-free and without signs of toxicity when the experiment was terminated 3 months later. In the fifth mouse, the tumor nodule decreased in size as long as the mouse received K1735-1D8 cells, but the tumor grew back after therapy was terminated.

In another experiment with 5 mice/group, mice were injected intravenously (i.v.) with  $3 \times 10^5$  K1735-WT cells to initiate lung metastases. Three days later, K1735-1D8 cells were transplanted s.c. This procedure was repeated once weekly for a month; control mice were injected with PBS. The experiment was terminated when one mouse in the control group died, 37 days after receiving the K1735-WT cells. At that time, lungs of the control mice each had >500 metastatic foci. In contrast, less than 10 metastatic foci were present in the lungs of the immunized mice.

In a third experiment, mixtures of K1735-WT cells and K1735-1D8 cells were injected into immunocompetent syngeneic C3H mice. Mice were injected subcutaneously with K1735-WT cells alone or with a mixture of  $2 \times 10^6$  K1735-WT cells and  $2 \times 10^5$  K1735-1D8 cells. Tumor growth was monitored at 5-day intervals.

## EXAMPLE 27

### EXPRESSION OF ANTI-4-1BB scFv IgG-CD80 FUSION PROTEIN ON THE CELL SURFACE OF SARCOMA CELLS

This Example demonstrates expression of an anti-CD137 scFv on the cell surface of a second type of tumor cell by transfecting a murine sarcoma cell line with an anti-CD137 scFv IgG-CD80 construct.

The 1D8 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO:     ) was transferred from the pLNCX vector into pCDNA3-hygro vector using restriction enzyme digestion and ligation steps according to standard molecular biology methods. The construct was cut with HindIII + ClaI and the scFv fragment was filled in with Klenow (Roche) and the blunt-ended fragment was ligated into EcoRI site of pCDNA3. Ag104 murine sarcoma tumor cells were transfected with the pCDNA3-hygro vector

containing the 1D8 scFv IgG CD80 fusion protein. Hygromycin-resistant clones were screened by flow cytometry using a FITC anti-human IgG antibody to detect expression of the transgene. Only approximately 15% of the resistant clones had detectable fusion protein initially. Positive cells identified by flow cytometry were repeatedly panned on flasks  
5 coated with immobilized anti-human IgG (10  $\mu$ g/ml) according to standard methods. Panning was performed by incubating cells on the coated plates for 30 min at 37C; the plates were then washed 2-3x in versene or PBS. After each round, cells were tested for IgG expression by FACS. The histogram in Figure 44 shows the staining pattern after four rounds of panning against anti-human IgG (black). Untransfected cells were stained and are  
10 indicated in gray. All of the cells in the population were positive.

## EXAMPLE 28

### **CONSTRUCTION AND CHARACTERIZATION OF A BISPECIFIC scFv Ig FUSION PROTEIN AND scFv Ig FUSION PROTEINS WITH A MUTATION IN THE IgG1 CH2 DOMAIN**

An anti-CD20 (2H7) scFv IgG fusion protein- was constructed that had a  
15 mutant hinge (MT (SSS)) and a mutant CH2 domain in which the proline at residue (position number 238 according to Ward *et al.*, *supra*) was substituted with a serine. The 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide (SEQ ID NO:\_\_) was constructed essentially according to methods described in Examples 1, 5, and 13. The IgG mutant hinge-mutant CH2-wild type CH3 domains were also fused to an anti-CD20 (2H7)-  
20 anti-CD40 (40.2.220) bispecific scFv. The anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 encoding polynucleotide sequence is shown in SEQ ID NO:\_\_\_ and the encoded polypeptide is shown in SEQ ID NO:\_\_\_.

COS cells were transiently transfected with vectors comprising the polynucleotide sequences encoding 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID  
25 NO:\_\_); anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3 (SEQ ID NO:\_\_); 2H7 scFv IgG MTH (SSS) WTCH2CH3 (SEQ ID NO:\_\_); and 2H7 scFv IgAH IgG WTCH2CH3 (SEQ ID NO:\_\_\_) as described in Example 10. Culture supernatants were collected and the fusion proteins were purified by protein A chromatography (see Example 10). The purified polypeptides were fractionated by SDS-PAGE according to the method

described in Example 10. Rituximab (anti-CD20 monoclonal antibody), and Bio-Rad prestained molecular weight standards (Bio-Rad, Hercules, CA), and Multimark® molecular weight standards (Invitrogen Life Technologies) were also applied to the gel. The results are presented in Figure 45.

5                   The 2H7 scFv Ig fusion protein that contains a mutation in the CH2 domain was compared to fusion proteins that have the wild type CH2 domain in an ADCC assay. The assays were performed essentially as described in Examples 11 and 19. Fresh resting PBMC (effector cells) were added to <sup>51</sup>Cr-labeled BJAB cells (target cells) at the ratios indicated in Figure 46. Purified 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, 2H7 scFv IgG  
10 MTH (SSS) WTCH2CH3, 2H7 scFv IgAH IgG WTCH2CH3, and Rituximab, each at 10 µg/ml were added to the effector/target cell mixtures and incubated for five hours at 37 °C. Supernatants were harvested and the amount of chromium released was determined as described in Examples 11 and 19. Percent specific killing by each fusion protein is presented in Figure 46.

15

## EXAMPLE 29

### **TUMOR CELL SURFACE EXPRESSION OF AN ANTI-HUMAN CD3 scFv IgG FUSION PROTEIN**

An anti-human CD3 scFv Ig CD80 fusion protein was prepared essentially as described in Examples 1 and 12. The fusion protein comprised an anti-human CD3 scFv  
20 fused to wild type IgG1 hinge (SEQ ID NO:\_\_) and wild type CH2 (SEQ ID NO:\_\_) and CH3 (SEQ ID NO:\_\_) domains, fused to CD80 transmembrane and cytoplasmic domains (SEQ ID NO:\_\_) to enable cell surface expression of the anti-CD3 scFv. The anti-human CD3 scFv IgG WTH WTCH2CH3-CD80 polynucleotide (SEQ ID NO:\_\_) encoding the polypeptide (SEQ ID NO:\_\_) was transfected in Reh cells and into T51 cells  
25 (lymphoblastoid cell line). Expression of the anti-human CD3 scFv IgG fusion protein was detected by flow cytometry using FITC conjugated goat anti-human IgG (see methods in Examples 4, 10, 16, 18). Figure 47A illustrates expression of the anti-human CD3 fusion protein on the cell surface of Reh cells, and figure 47B shows expression of the fusion protein on T41 cells.



ADCC assays were performed with the transfected Reh and T51 cells to determine if expression of the scFv-Ig polypeptides on the cell surface augmented effector cell function. Untransfected and transfected Reh cells and untransfected and transfected T51 cells were pre-labeled with  $^{51}\text{Cr}$  (100  $\mu\text{Ci}$ ) (Amersham) for two hours at 37 °C. Human PBMC served as effector cells and were added to the target cells ( $5 \times 10^4$  cells per well of 96 well plate) at ratios of 20:1, 10:1, 5:1, and 2.5:1. After four hours at 37 °C, culture supernatants were harvested and analyzed as described in Examples 11 and 12. Percent specific killing was calculated as described in Example 12. The results are presented in Figure 48.

### EXAMPLE 30

#### INDUCTION OF CYTOKINE EXPRESSION IN TUMOR CELLS EXPRESSING

##### ANTI-CD28 scFV ON THE CELL SURFACE

This Example describes the effect of cell surface expressed scFv on cytokine mRNA induction in stimulated lymphocytes co-cultured with tumor cells transfected with an anti-human CD28 scFv IgG-CD80 fusion protein.

Real time PCR analysis was performed on RNA samples from human PBMC stimulated with Reh, Reh-anti-CD28 (2e12) (see Example 12 for construction of 2e12 scFv IgG WTH WHTCH3CH2-CD80 and transfection of Reh cells), and Reh-CD80 (see Example 14) in order to measure the effects of the surface expressed scFv on cytokine production by the PBMC effector cells. For the real-time PCR assay, SYBR Green (QIAGEN) (Morrison *et al.*, *Biotechniques* 24:954-8, 960, 962 (1998)) was used and measured by an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) that measures the formation of PCR product after each amplification cycle. Cells were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA. cDNA was reverse transcribed using equal amounts of RNA from each cell type and Superscript II Reverse Transcriptase (Life Technologies). SYBR Green real-time PCR analysis was then performed using the prepared cDNA as template and primer pairs specific for cytokine gene products. The average length

of the PCR products that were amplified ranged from 150-250 base pairs. The cDNA levels for many activation response molecules including IFN $\gamma$ , TNF $\alpha$ , GM-CSF, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-15, ICOSL, CD80 and CD86 were assayed. Control reference cDNAs for constitutively expressed genes, including GAPDH,  $\beta$ -actin, and CD3 $\epsilon$  were measured in each assay. The most significant induction of specific mRNA was observed for IFN- $\gamma$ , and more modest induction was observed for CTLA-4 and ICOS.

### EXAMPLE 31

#### **CLONING OF AN ANTI-HUMAN 4-1BB ANTIBODY AND CONSTRUCTION OF AN ANTI-HUMAN 4-1BB scFv IG FUSION PROTEIN**

10 A hybridoma cell line expressing a mouse anti-human monoclonal antibody (designated 5B9) was obtained from Dr. Robert Mittler, Emory University Vaccine Center, Atlanta, GA. The variable heavy and light chain regions were cloned according to known methods for cloning of immunoglobulin genes and as described herein. Cells were grown in IMDM/15% FBS (Invitrogen Life Technologies) media for several days. Cells in logarithmic growth were harvested from cultures and total RNA prepared using QIAGEN RNA kits, including a QIA shredder column purification system to homogenize cell lysates, and RNeasy® mini-columns for purification of RNA according to manufacturer's instructions. cDNA was reverse transcribed using random hexamer primers and Superscript II Reverse Transcriptase (Invitrogen Life Technologies).

20 cDNA was anchor-tailed using terminal transferase and dGTP. PCR was then performed using an anchor-tail complementary primer and a primer that annealed specifically to the antisense strand of the constant region of either mouse Ck (for amplification of VL) or the appropriate isotype mouse CH1 (for amplification of VH). The amplified variable region fragments were TOPO® cloned (Invitrogen Life Technologies), and clones with inserts of the correct size were then sequenced. Consensus sequence for each variable domain was determined from sequence of at least four independent clones. The 5B9 VL and VH polynucleotide sequences are shown in SEQ ID NOs:\_\_\_ and \_\_\_, respectively, and the deduced amino acid sequences are shown in SEQ ID NOs:\_\_\_ and \_\_\_. The scFv was constructed by a sewing PCR method using overlapping primers containing a

synthetic (gly<sub>4</sub>ser)<sub>3</sub> linker domain inserted between the light and heavy chain variable regions (see Example 1). The 5B9 scFv polypeptide (SEQ ID NO:\_\_) is encoded by the polynucleotide sequence comprising SEQ ID NO:\_\_\_.

5B9 scFv polynucleotide sequence was fused in frame to the polynucleotide sequence encoding the human IgG1 mutant hinge and wild type CH2 and CH3 (MTH (SSS) WTCH2CH3, SEQ ID NO:\_\_\_) according to methods described in Examples 5, 10, and 13. COS cells were transiently transfected with a vector comprising the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polynucleotide sequence (SEQ ID NO:\_\_\_). Supernatant was collected and binding of the 5B9 scFv IgG MTH (SSS) WTCH2CH3 polypeptide (SEQ ID NO:\_\_\_) was measured by flow immunocytofluorimetry essentially as described in Examples 4, 10, 16, and 18. Culture supernatant from the 5B9 hybridoma cell line was also included in the binding assay. Fresh human PBMC were incubated in the presence of immobilized anti-CD3 for four days prior to the binding experiment to induce expression of CD137 on the surface of activated T cells. Stimulated PBMC were washed and incubated with COS or hybridoma culture supernatant containing the 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody, respectively, for 1 hour on ice. Binding of 5B9 scFv IgG fusion protein or 5B9 murine monoclonal antibody was detected with FITC conjugated anti-human IgG or anti-mouse IgG, respectively. The results are presented in Figure 49.

#### EXAMPLE 32

##### 20 CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

A 2H7 scFv IgG fusion proteins are constructed with the first cysteine residue and the second cystein in the IgG1 hinge region substituted with a serine residue to provide MTH (SCC) and MTH (CSC). The template for introduction of the mutations is a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO:\_\_\_). The oligonucleotide introducing the mutations are 5' PCR primer oligonucleotides HIgGMHcys1 (SEQ ID NO:\_\_\_) and HIgGMHcys2 (SEQ ID NO:\_\_\_). The constructs are prepared as described in SEQ ID NO:\_\_\_). The encoding polynucleotides of the mutants are presented in SEQ ID NOs:\_\_\_) and the polypeptide sequences are provided in SEQ ID NO:\_\_\_).

### Example 33

#### CONSTRUCTION OF 2H7 V<sub>H</sub>L11S scFv (SSS-S) H WCH2 WCH3

A change from leucine to serine at position 11 in the heavy chain variable region (numbering according to Kabat et al., *Sequences of Proteins of Immunological Interest*, 5<sup>th</sup> ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)) was introduced into the 2H7 scFv MTH (SSS) WTCH2CH3 fusion protein (SEQ ID NO:\_\_\_). The wild type leucine residue was substituted with serine by site-directed mutagenesis using the oligonucleotide Vhser11: 5'-gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc-3' (SEQ ID NO:\_\_\_). The 3'-primer for PCR was huIgG1-3' having the sequence 5'-gtc tct aga cta tca tt acc cgg aga cag-3' (SEQ ID NO:\_\_\_) (XbaI site underlined and italicized). After PCR amplification, the fragments were inserted into the TOPO® cloning vector and sequenced to confirm the presence of the VH11 leucine to serine mutation. The 2H7 scFv-IgG (SSS-S) H WCH2 WCH3 encoding DNA was shuttled into the PSL1180 cloning vector (Pharmacia Biotech, Inc., Piscataway, NJ). The construct PSL1180-2H7 scFv-IgG (SSS-S) H WCH2 WCH3 was digested with Sac and XbaI to remove the wild type VH domain and the connecting region and CH2 and CH3 domains. The PCR product comprising the VH11 mutant was digested with Sac and XbaI and then inserted into the digested PSL1180 construct according to standard molecular biology procedures. The construct was then digested with Hind III and XbaI, and inserted into the mammalian expression vector pD18 (see methods described in Example 1 and Example 10). The mutant is designated 2H7 scFv VH L11S IgG (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

### EXAMPLE 34

#### EXPRESSION AND OF 2H7 scFv VH L11S (SSS-S) H WCH2 WCH3

##### IN STABLE CHO LINES

CHO DG44 cells were transfected by electroporation with approximately 150 micrograms of linearized expression plasmid encoding the 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3. Cultures were plated in selection media containing 100 nM methotrexate, in

96 well, flat bottom tissue culture plates at various numbers of cells/well, ranging from 125 to 2000. Methotrexate resistant clones were selected and culture supernatants were screened for the highest expressors of the fusion protein using a CD20CHO binding assay similar to that described for Figure 1. Clones were amplified after the initial selection in gradually increasing doses of methotrexate. Cells were passaged for two passages in the higher concentration prior to adjusting the concentration to the next higher dose. Clones were amplified to a final concentration of 1 micromolar methotrexate.

Figure 50B illustrates the production levels of 2H7 VH L11S scFv (SSS-S)H WCH2 WCH3. Spent supernatants from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were tested for quantitative binding to CD20 CHO cells by flow cytometry. The activity was converted to protein concentration by generation of a standard curve using the same molecule purified from supernatants with Protein A affinity chromatography (Figure 50A). The concentration of the purified protein was determined by A280 using an extinction coefficient provided by the amino acid composition of the recombinant protein (Vector NTI). Although levels of production varied between clones tested, multiple clones produced over 1 mg/ml. This level of protein expression is over 10-fold higher than the identical molecule except for the amino acid change in V<sub>H</sub>.

Figure 51 illustrates the production levels of 2H7 VH L11S scFv (SSS-S) H WCH2 WCH3 by semi-quantitative analysis on SDS-PAGE. Ten microliters of spent supernatant from amplified CHO cells expressing this molecule and growing in stationary T25 flasks were mixed with 10 microliters 2X non-reducing SDS sample buffer, run on SDS-PAGE gels, and stained with coomassie blue.

### EXAMPLE 35

#### CONSTRUCTION AND BINDING CAPACITY OF G28-1 scFv Ig CONSTRUCTS

Construction of the G28-1 (anti-CD37) scFv was performed using total RNA isolated from the G28-1 hybridoma using Trizol (Invitrogen) reagent according to manufacturer's instructions. cDNA was prepared using random primers and the protocol described previously for 2H7 cloning in Example 1. The variable domains of the scFv was cloned using one of two methods: the first method used a family of degenerate 5'

oligonucleotides specific for each V region gene family and a single 3' primer specific for the constant region of either the light or heavy chain using methods and primers described in (Ig-Prime Kit Mouse Ig-Primer Set, Novagen). The second approach used the anchor-tailing methods and primers described in (Gilliland LK et al, Tissue Antigens 47: 1-20 (1995). In either case, PCR amplified products were cloned into the TOPO cloning vector (Invitrogen). The clones were digested with EcoRI and screened for inserts of the proper size. Positive clones were sequenced as previously described in Example 1.

Specific primers were then designed for each V region, one with the leader sequence and one without. Primers were also designed to include desired linkers and/or restriction sites at the primer ends. PCR reactions were performed on the TOPO cloned DNA using a 25 cycle program with the following profile: 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, followed by a final extension at 72C for 8 minutes. PCR products were gel purified and fragments recovered using a QIAQUICK gel extraction kit (QIAGEN, Valencia, CA). Fragments were diluted 1:50 and 1 microliter used for SEWING PCR reactions according to the methods described in Example 1. The following oligonucleotides were used for the secondary PCR reactions of the V<sub>L</sub> domain for the G28-1 scFv:

5' primers with SalI site without leader: 5'-GTTGTTGTGCGACATCCAGATGACTCAGTC TCCA-3' (SEQ ID NO\_\_)

5' primer with HindIII site and leader sequence: 5'-

GTCAAGCTTGCCGCCATGGTATCCA  
CAGCTCAGTTCCTTGG-3' (SEQ ID NO\_\_)

3' primer: 5'-

GCCACCCGACCCACCAACCGCCCGAGCCACCGCCACCTTTGATCTCCA  
GTTCGGTG CC-3' (SEQ ID NO\_\_)

The primers used for the V<sub>H</sub> domain are shown below:

5' sense primer:

TCGGGCGGTGGTGGGTGCGGTGGCGGCGGATCGTCAGCGGTCCAGCTGCA  
GCAGTCTGGA-3' (SEQ ID NO\_\_)

3' antisense primer with BclI site: 5'-

TCAGTGCTGATCAGAAGAGACGGTGACTGAGGTTTCCTTG-3' (SEQ ID NO\_\_).

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the G28-1 scFv by site-directed mutagenesis. The wild type form of the G28-1 scFv was initially constructed by sewing/overlap PCR to insert a (gly4ser)<sub>3</sub> linker between the VL and VH domains as described above. However, no Sac I site was introduced as a part of this fusion of the variable domains, so alternative, nearby restriction sites (HaeII and PvuII) near leucine 11 were used to synthesize the VL+mutated VH domain. Primers were designed to contain one of these sites and the DNA sequence including the L to S change, followed by 12 wild type base pairs. Several attempts at this strategy failed, so an alternative strategy using the Genetailor (Invitrogen) method of site directed mutagenesis was used to introduce the desired mutation. The mutagenesis was carried out according to manufacturer's instructions. Briefly the procedure involves methylation of the plasmid DNA with DNA methylase, amplification of the DNA in a mutagenesis reaction with two overlapping primers, one of which contains the target mutations, transformation of the plasmid into wild type E.coli which digests all methylated DNA and leaves only the unmethylated, mutated amplification product. Both primers are approximately 30 nucleotides in length (not including the mutation site on the mutagenic primer, with an overlapping region at the 5' ends of 15-20 nucleotides, for efficient end-joining of the mutagenesis product. The template for the mutagenesis reaction was 100 ng of a plasmid containing the wild type G28-1 scFvIg construct, and the primers used for the G28-1 VH mutagenesis are as follows:

Forward primer:

5'-GCAGCAGTCTGGACCTGAGTCGGAAAAGCCTG-3' (SEQ ID NO\_\_)

Reverse Primer:

5'-CTCAGGTCCAGACTGCTGCAGCTGGACCGC-3' (SEQ ID NO\_\_)

PCR reactions were performed using the 15 ng methylated template, the primers above, and the usual reaction components as previously described. A 20 cycle program with the following profile was used for amplification: 94C, 30 sec; 55C, 30 sec; 68C, 8 min, followed by a final 68C extension step for 10 minutes. PCR products were transformed into wild type bacteria, and colonies screened by sequencing. Clones with only the desired mutation were isolated and plasmid prepared as previously described Example

33. The mutant is designated G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

5 The expression level of G28-1 fusion proteins was confirmed using Immunoblot analysis according to the methods described in Example 17. Figure 53 illustrates a large increase in protein expression in the VH L11S mutant G28-1 fusion proteins compared to the G28-1 fusion protein without the mutation.

10 The G28-1 scFv Ig fusion proteins were transiently transfected and expressed in COS cells according to methods described in Example 10. Figure 52 illustrates the capacity of the G28-1 scFv Ig fusion proteins from the COS supernatants to bind CD37. Ramos and BJAB cells both express human CD37, and were therefore used to screen the G28-1 supernatants for functional activity. Binding of G28-1 scFv (SSS-S) H WCH2 WCH3 and G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 to CD37+ Ramos cells was measured by flow cytometry according to methods described in Example 2. Each point on the graph represents the mean of five replicate transfections. The graph illustrates that the G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3 is able to bind CD37+ Ramos cells.

20 Addition constructs were made with different connecting regions. The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with BclI and XbaI to remove the connecting region, CH2 and CH3. These were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13. The new constructs were designated: G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO\_\_), G28-1 scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO\_\_), G28-1 scFv VHL11S (CSS-S) H WH2 WH3 (SEQ ID NO\_\_), G28-1 scFv VHL11S (SSC-P) H WH2 WH3 (SEQ ID NO\_\_), G28-1 scFv VHL11S (SCS-S) H WH2 WH3 (SEQ ID NO\_\_), G28-1 scFv VHL11S (CCS-P) H WH2 WH3 (SEQ ID NO\_\_), and G28-1 scFv VHL11S (SCC-P) H WH2 WH3 (SEQ ID NO\_\_).

25 The G28-1 scFv was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4. The pD18 G28-1 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3. The IgA regions were inserted using methods described in Example 13. The construct was



designated G28-1 scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO\_\_). The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39. The construct was designated G28-1 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO\_\_).

5

### EXAMPLE 36

#### **Characterization of 2H7 scFv Ig Mutant Fusion Proteins**

Figure 54 illustrates the binding capacity of purified 2H7 scFv Ig constructs to CD20+ CHO cells. The proteins were transfected into stable CHO cells according to methods described in Example 2. Binding was determined using flow cytometry according to the methods described in Example 2. The graph in Figure 54 illustrates that these proteins retain binding function to CD20 with altered connecting regions. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted).

The ability of 2H7scFv-Ig constructs with mutated connecting regions to kill CD20 positive cells in the presence of peripheral blood mononuclear cells (PBMC) through ADCC was tested by measuring the release of <sup>51</sup>Cr from labeled BJAB cells in a 4 hr. assay using 100:1 ratio of PBMC to BJAB cells. The results shown in Figure 55 indicate that 2H7scFv-Ig mutants can mediate antibody dependent cellular cytotoxicity (ADCC), since the release of <sup>51</sup>Cr was significantly higher in the presence of both PBMC and 2H7scFv-Ig than in the presence of either PBMC or 2H7scFv-Ig alone. Comparative results were obtained in each of the 2H7 scFv VHL11S mutants with each type of altered connecting region (results omitted).

The ability of 2H7scFv-Ig mutant fusion proteins to kill CD20 positive cells in the presence of complement was tested using B cell lines Ramos target cells. Rabbit complement was purchased from Pel-Freez (Rogers, AK), and was used in the assay at a final concentration of 1/10. Purified 2H7scFv-Ig was incubated with B cells and complement for 45 minutes at 37°C, followed by counting of live and dead cells by trypan blue exclusion. The results in Figure 56 show that 2H7scFv-Ig mutants in the presence of rabbit complement, lysed B cells expressing CD20.

**EXAMPLE 37**  
**COMPARATIVE BINDING OF IGA, IGG, AND IGE 2H7 scFv CONSTRUCTS**

Binding capacity of Ig constructs IgA, IgG and IgE were measured using flow cytometry according to the methods described in Example 2, using a commercially available (Caltag) second step specific for each Ig tail. The results in Figure 57 show that all the IgE constructs were able to bind CD20+ CHO cells comparable to the binding abilities of IgG and IgA. These results also demonstrate that the IgE constructs were detected with the IgE second step, but not the IgA or IgG second step.

**EXAMPLE 38**  
**CONSTRUCTION AND CHARACTERIZATION OF 2H7 VH L11S IGE CONSTRUCTS**

IgE tail RNA was isolated from SKO-007 cells(ATCC) using QIAGEN QIAshredder homogenization and RNA minikits. Random-primed cDNA was generated according to the usual protocol, with 4 microliters RNA eluted from the QIAGEN columns. Human IgE from the beginning of CH1 through CH4 (approximately 1.2 kb) was isolated by PCR amplification of 5 microliters cDNA, with an amplification profile of 94C, 60 sec; 72C, 2 minutes for 35 cycles, and the following primers:

5' primer: 5'-ggatccacccgctgctgcaaaaacattccctccaatgccacctccgtgac-3' (SEQ ID NO\_\_)

3' primer: 5'-tcatttacgggatttacagacaccgctcgtcgtggacggtctgtgaggggctcgtgc-3' (SEQ ID NO\_\_)

PCR fragments were ligated into PCR 2.1-TOPO vector, and transformants screened for inserts of the correct size by digestion with EcoRI according to the methods described in Example 1. One of the clones with the correct sequence from was used as template to amplify the CH2-CH4 domains with appropriate restriction sites attached for subcloning as soluble or cell surface (ORF) forms. The following primers were used with an amplification profile of 94C, 60 sec; 55C, 60 sec; 72C, 2 min; for 35 cycles to amplify a fragment of approximately 950 bp:

5' primer: (attaches BclI site to 5' end of CH2 domain of IgE)

5'-gttgttgatcacgtctgctccagggacttcacc-3' (SEQ ID NO\_\_)

3' primer: (attaches stop codon and XbaI site to 3' end of CH4 of IgE)

5'-gttgtttctagattatcattaccaggatttacagacaccgctcgtg-3' (SEQ ID NO\_\_)

3' primer: (attaches SfuI and BamHI to 3' end of CH4 without a stop codon)

5'-gttgttttcgaaggatccgctttaccagatttacagacaccgctcgtg-3' (SEQ ID NO\_\_)

The IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 IgECH2CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_. The IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 IgECH2CH3CH4(ORF). The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

The human IgE was also amplified as a fragment missing both CH1 and CH2 domains, with only the CH3 and CH4 domains attached to the human IgG1 hinge. Sequential PCR reactions using overlapping 5' oligonucleotides were used to attach the IgG1 hinge to the CH3 domain of human IgE. Primers for the first step of the PCR reaction:

5' Primer: 5'-actcacacatccccaccgtccccagcatccaacccgagaggggtgagc-3' (SEQ ID NO\_\_)

Primers for the second step of the PCR reaction:

5' primer: 5'-tctgatcaggagcccaaattcttgacaaaactcacacatccccaccg-3' (SEQ ID NO\_\_)

3' primer: 5'-gttgtttctagattatcattaccaggatttacagacaccgctcgtg-3' (SEQ ID NO\_\_)

The PCR product was digested with EcoRI and sequenced according to the methods described in Example 1. Positive clones were inserted into pD18 plasmid containing 2H7 VHL11S scFv (SSS-S) H. The construct was designated 2H7 VHL11S scFv (SSS-S) H IgE CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

Binding capacity of 2H7 scFv VH L11S IgECH2 CH3 CH4, was measured using flow cytometry, essentially according to Example 2. The protein was purified using MEP HyperCel, (Cipergen, Catalog # 12035-010, Lot# 200920/0271) chromatography resin and Hydrophobic charge induction chromatography (HCIC). HCIC absorbent is a high capacity, high selectivity, absorbent designed for capture and purification of monoclonal and polyclonal antibodies from various sources including cell culture supernatants. Columns were packed with a 10 ml bead volume of MEP Hypercel, and equilibrated with PBS, pH

7.4 containing 0.1% NaN<sub>3</sub>. Approximately 1 liter of 2H7 scFv VHL11S IgE CH<sub>2</sub>CH<sub>3</sub>CH<sub>4</sub> CHO culture supernatant was then run over the column. A series of citrate buffers ranging from pH 3-6 were prepared for elution of the fusion protein. The column was washed in PBS. Protein was eluted in fifteen 1 ml fractions at pH6, 5, and 4. A final 15 ml fraction was collected at pH 3.5. Aliquots from each fraction were analyzed for A280 and were also subjected to SDS-PAGE, loading roughly 10 micrograms/well based on the A280 reading. The results of these two analyses indicated that the bulk of the protein did not elute in citrate buffers at the higher pH, but eluted at pH4, and the post elution wash at pH3.5 also contained significant amounts of protein.

The ability of these 2H7 VH L11S IgE purified proteins to bind CD20+ CHO cells was determined using flow cytometry according to the methods described in Example 2 using FITC-conjugated goat-anti-human IgE. Figure 58A illustrates that both purified proteins are able to bind CD20+ CHO cells.

The ability of these 2H7 VH L11S IgE purified proteins to mediate ADCC against BJAB target cells with PBMC effectors was measured according to the methods described in Example 2. Figure 58B illustrates that both proteins were able to mediate ADCC at similar levels.

### EXAMPLE 39

#### **CONSTRUCTION AND BINDING CAPACITY OF SCFV VH L11S MUTANTS WITH MOUSE IGA AND IGE TAIL REGIONS**

Murine IgA was cloned from murine spleen RNA using essentially the same methods used to clone the human IgE tails in Example 38. The PCR reactions were performed with a 94C 60 sec; 52C 60 sec; 72C 2 min amplification profile for 35 cycles. The PCR primers used to clone CH1-CH4 regions were:

5' primer: 5'-atctgttctctctactactctctccacct-3' (SEQ ID NO\_\_)

3' primer: 5'-tcagtagcagatgccatctccctctgacatgatgacagacagct-3' (SEQ ID NO\_\_)

PCR primers used to delete the CH1 region:

5' primer: 5'-gttgatcacatctgttctctctactactctctccacct-3' (SEQ ID NO\_\_)

3' primer with a stop codon, XbaI site at end of Ig tail, and the T4 mutation in CH3 region:

5'-gttgtttctagattatcaatctccctctgacatgatgacagacac-3' (SEQ ID NO\_\_)

3' primer for the ORF, a SfuI and BamHI sites, and T4 mutation in the CH3 region:

5'-gttcttcgaaggatccgcatctccctctgacatgatgac-3' (SEQ ID NO\_\_)

5 The mouse IgACH2 T4CH3 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv and the IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

10 The mouse IgACH2 T4CH3 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted in into a pD18 vector that contains 2H7 VHL11S scFv and IgAH. This construct was designated 2H7 VHL11S scFv IgAH mIgACH2 T4CH3 (ORF). The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

15 Murine IgE was cloned murine IgE La2 (ATCC) RNA essentially according the methods described in Example 38. The PCR reactions were performed with a 94C 60 sec; 52C 60 sec; 72C 2 min amplification profile for 35 cycles. Initial PCR primers used to clone the CH1-CH4:

5' primer: 5'-tctatcaggaaccctcagctctacccttgaagccctg-3' (SEQ ID NO\_\_)

3' primer: 5'-gttgtttctagattatcaggatggacggaggagggtgtaccaaggct-3' (SEQ ID NO\_\_)

20 PCR primers to remove the CH1 region:

5' primer: 5'-gttggtgatcacgttcgacctgtcaacatcactgagcccacc-3' (SEQ ID NO\_\_)

3' primer with stop codon and XbaI site:

5'-gttgtttctagattatcaggatggacggaggagggtgtaccaaggct-3' (SEQ ID NO\_\_)

25 3' primer ORF, SfuI and Bam HI: 5'-gttgtttctgaaggatccgcggatggacggaggagggtgtta-3' (SEQ ID NO\_\_)

The mouse IgE CH2CH3CH4 tail with a stop codon was digested with BclI and XbaI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 VHL11S mIgECH2CH3CH4. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

The mouse IgE CH2CH3CH4 tail with no stop codon (ORF) was digested with BclI and SfuI and inserted into a pD18 vector that contains 2H7 VHL11S scFv. This construct was designated 2H7 VHL11S scFv mIgECH2CH3CH4(ORF). The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

Binding capacity of 2H7 VH L11S mIgE and mIgA (with mouse tail regions) to CD20+ CHO cells were also measured by flow cytometry according to the methods described in Example 2 using commercially available IgE or IgA second step reagents (Caltag). Each point in Figure 59 represents the mean of a population with brightness corrected by subtracting the binding of the second step alone. This figure illustrates that these constructs have the ability to bind CD20+ CHO cells.

#### EXAMPLE 40

##### **HPLC PROFILES OF 2H7 scFv Ig MUTANT FUSION PROTEINS**

HPLC analysis of purified 2H7 scFv-Ig mutant fusion proteins with altered connecting and CH3 regions. Each protein was purified by Protein A affinity chromatography from supernatants of transfected COS or CHO cells. Twenty-five to fifty micrograms of each sample was run at 1 ml/min in PBS on a TSK-GEL G3000SW<sub>XL</sub> 30 cm column (Tosoh Biosep, Stuttgart, Germany). The arrow near the beginning of each profile represents the sample injection point. Gel filtration standards (Bio-Rad) included thyroglobulin (670 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (12.5 kDa), and vitamin B-12 (1.35 kDa). Standards were run at the beginning and end of each experiment. Migration positions of standards are shown and did not vary between experiments. (Figures 60-62)

#### EXAMPLE 41

##### **BINDING CAPACITY OF 2H7 VH L11S MUTANT FUSION PROTEINS**

Binding effects of the CH3 mutant were compared to the non-mutated CH3 fusion protein. The constructs were transfected into COS cells and purified from the supernatant using protein A column purification techniques described in Example 2. Figure 63 illustrates the differential effects of CH3 mutations on binding using flow cytometry

according to the methods described in Example 2. This figure illustrates that some binding ability is lost when the double point mutation is introduced in the CH3 region.

Binding of fluorescein isothiocyanate (FITC) conjugated 2H7 VH L11S mutant fusion proteins was determined using flow cytometry. A 1 mg/ml solution of FITC was prepared in DMSO. Fusion proteins were dialyzed in pH 9.3 bicarbonate buffer overnight at 4C in a volume of 2 liters. Concentration of the protein was adjusted to 1-5 mg/ml prior to conjugation. A series of Falcon 5 ml tubes was set up with varying FITC to protein ratios, ranging from 15-60, but minimally ratios of 20 and 40. Conjugation reactions were incubated at 37C for 30 minutes protected from light. Fluorescing labeled protein was separated from free fluorescein on a 2 ml Sephadex G-25 column equilibrated with PBS, 0.5 M NaCl, and 1% NaN<sub>3</sub>. The fluorescein labeled protein eluted from the column first and was collected in a 5ml tube. The degree of labeling was determined by measuring the absorbance of the diluted conjugate at 280 and 494 nm, and utilizing the formulas provided by technical services at Molecular Probes (Eugene, OR). Data has been corrected for FITC: protein ratio. Figure 64 illustrates that these constructs do not lose binding capacity when conjugated to a fluorescent marker.

Purified 2H7 VH L11S constructs were run on a non-reducing SDS gel according to the methods described in Example 2. The migration patterns are presented in Figure 65.

#### EXAMPLE 42

##### CHARACTERIZATION OF 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 IN Lec13 CHO CELLS

2H7 scFv VH L11S (CSC-S) H WCH2 WCH3 were transiently transfected and expressed in Lec13 CHO cells. Lec13CHO cells were used as the mammalian cell hosts for either the 2H7 scFv VHS11 hIgG1 (CSS-S)H WCH2 WCH3 and (CSC-S)H WCH2 WCH3 expressing plasmids in side-by-side transfections. All transfections were performed in 100 mm tissue culture dishes. Cells were transfected when approximately 90% confluent using lipofectamine 2000 (Invitrogen, Catalog #: 11668-027, 0.75 ml), following manufacturer's instructions. Both cell lines were grown in the presence of serum to promote

and maintain adherence to the cell culture dishes, simplifying transfection manipulations, washes, and supernatant harvests. DNA: lipofectamine complexes were allowed to form in the absence of serum and antibiotics, following the suggested protocol /conditions recorded in the product insert. Culture supernatants were harvested 72 hours after transfection, and then again 72 hours after the first harvest. Fusion protein from the two CHO sources was isolated by protein A purification as previously described and used in CD20 binding and ADCC assays.

The ability of mutated fusion protein to mediate ADCC in CD20 positive cells was determined using the methods described in Example 2. Constructs expressed in Lec13 CHO cells exhibited better binding to CD20 CHO target cells and also showed significantly improved activity in ADCC assays relative to the CHO DG44 derived proteins at equivalent concentrations as illustrated in Figure 67.

### EXAMPLE 43

#### CONSTRUCTION OF HIGH AND LOW AFFINITY CD16 ALLELES

The low(V) and high(F) affinity alleles at position 158 of the human CD16 extracellular domain were cloned from cDNA derived from human PBMC using PCR assay. PCR reactions used random primed cDNA made from PBMC stimulated for 3 days with immobilized anti-CD3 antibody (64.1) prior to harvest. PCR reactions included 2, 4, 6 or 8 microliters of cDNA, each primer at 25 pmol, and an amplification profile of 94C 60 sec; 55C 60 sec; 72C 2 min, for 35 cycles. The PCR primers are listed below:

5' primer - no leader peptide: 5'-GTTGTTACCGGTGCAATGCGGACTGAAGATCTCCC  
AAAGGCTGTG-3' (SEQ ID NO\_\_)

3'antisense primer: 5'-

GTTGTTTGATCAGCCAAACCTTGAGTGATGGTGATGTTTCA-3' (SEQ ID NO\_\_)

Positive clones were sequenced, and inserted into a vector containing an efficient leader peptide and the (SSS-S)H P238SCH2 WCH3 human IgG tail. Two different versions of the CD16 ED fusion proteins were expressed. The first contained the F158 (high affinity) and the second contained the V158 (low affinity) allele. Constructs were cloned into a (SSS-S)H P238S CH2 WCH3 pD18 plasmid and expressed in COS and CHO cells as



previously described in examples 1 and 10. CHO clones were screened for expression using an IgG sandwich ELISA to determine relative expression levels of the fusion proteins in the culture supernatant using the following protocol: Immulon 1V plates were coated at 4C with 0.4 microgram/ml goat anti-human IgG (mouse Adsorbed), (CalTag, Catalog # H10500) in PBS buffer. Plates were then blocked in PBS/1.5% nonfat milk at 4C overnight. Plates were washed three times in PBS/0.1% Tween 20, then incubated with 100 microliters dilution series from CHO clone culture supernatants at room temperature for 3 hours. Four dilutions per clone were added to successive wells, diluting in 5 fold increments from 1:5 to 1:375. In addition a standard curve was derived using CTLA4 hIgG1 (SSS-S)H P238SCH2 WCH3 as a concentration standard. The dilution series utilized 5 fold dilutions starting at 0.5 micrograms/ml; a second set of 8 wells was used to make a 2-fold dilution series starting at 0.34 micrograms/ml. Plates were washed 3 times in PBS/0.1% Tween 20, and incubated with goat anti-human IgG, conjugated to horseradish peroxidase (GAH IgG-HRP) at 1:5000, in PBS/0.5% BSA for 1 hour. Plates were washed four times with PBS/0.1% Tween 20, then TMB chromagen substrate (BD-Pharmingen) was added for 10 minutes, and reactions stopped by addition of 100 microliters 1N sulfuric acid. Plates were then read at 415nm on a SpectraCount plate reader. Concentrations of fusion protein were estimated by comparison of the ODs in the linear range to the CTLA4Ig standard curve run on each plate.

Proteins were purified using Protein A purification and were directly conjugated to fluorescein isothiocyanate (FITC) as described in Example 42. These proteins were run out on SDS gels under reduced and nonreduced conditions according to the methods described in Example 2. The migration of these proteins is presented in Figure 68.

The ability of the high and low CD16 alleles to bind 2H7 VH111S (CSC-S) H WCH2 WCH3 or bind 2H7 VH111S (SSS-S) H (P238S)CH2 WCH3 expressed on the cell surface of CD20+ CHO target cell is determined using flow cytometry according to the methods described in Example 2. The results in Figure 69 demonstrate both the high and low affinity alleles were able to bind 2H7 VHL11S (CSC-S)H WCH2 WCH3 (SEQ ID NO- ) and lost some binding capabilities when the P238S mutation was introduced into the CH2 region of the construct (SEQ ID NO- ).

#### EXAMPLE 44

##### MAMMALIAN DISPLAY SYSTEM

Figure 70A diagrams how FITC conjugates of FcRIII (CD16) soluble fusion proteins bind to 2H7 scFv-Ig constructs that are attached to CD20 expressed by CHO cells.

- 5 The CD16 binding to a scFv-Ig provides a screening tool for detecting changes in CD16 binding to an altered scFv-Ig constructs containing targeted or site-specific mutations. Changes in CD16 binding properties may be changes in binding of either CD16 high affinity protein (158F) or CD16 low affinity protein (158 V) or both.

- A schematic representation of such a screening process is diagrammed in  
10 Figure 70B, where scFv-Ig constructs are displayed on the cell surface of mammalian cells. The scFv-Ig molecules in this example are displayed on the cell surface because they contain a transmembrane domain anchor. These molecules may represent a single scFv-Ig construct or may be introduced into a population of mammalian cells as a library of such molecules. Transfected cells with altered binding properties can then be panned, sorted, or  
15 otherwise isolated from other cells by altering the stringency of the selection conditions and using CD16 fusion proteins as the binding probe. Cells that express scFv-Ig molecules with altered binding to either CD16 high affinity allele (158F) or CD16 low affinity allele (158V) or both can be isolated. For example, this display system can be used to create a library of mutated Ig tails with short stretches of CH2 sequence replaced with randomized  
20 oligonucleotides or possibly randomization of a single residue with all possible amino acid substitutions, including synthetic amino acids. Once such a library is constructed, it can be transfected into COS cells by methods well known in the art. Transfectants can then be bound to the labeled CD16 constructs, and panned or sorted based on their relative binding properties to multiple allelotypes/isoforms. Panned cells are harvested, and the plasmid  
25 DNA is isolated and then transformed into bacteria. This process may be repeated iteratively multiple times until single clones are isolated from the mammalian host cells (see Seed B and Aruffo A, PNAS 84:3365-3369 (1987) and Aruffo A and Seed B, PNAS 84: 8573-8577 (1987) ). One such use of this type of screening system would be to isolate Ig tails which bind equally well to both the high and low affinity alleles of CD16 with the goal  
30 of improving effector functions mediated by scFv-Ig constructs in multiple subpopulations

of patients. Ig tails with altered binding properties to other Fc receptors can also be selected using the display system described. Other display systems for example those that use bacteriophage or yeast are not suitable for selection of Ig tails with altered FcR binding properties because of the requirement for glycosylation in the Ig CH2 domain that would not occur in non-mammalian systems.

This system is also useful for selection of altered scFv-Ig molecules that will be produced at higher levels. In this example, mammalian cells such as COS cells can be transfected with a library of scFv-Ig constructs in a plasmid that directs their expression to the cell surface. COS cells that express the highest levels of the scFv-Ig molecules can be selected by techniques well known in the art (for example panning, sterile cell sorting, magnetic bead separation, etc), and plasmid DNA is isolated for transformation into bacteria. After several rounds of selection single clones are isolated that encode scFv-Ig molecules capable of high level expression. When the isolated clones are altered to remove the membrane anchor and then expressed in mammalian cells, the scFv-Ig constructs will be secreted into the culture fluid in high levels. This reflects the common requirement of secreted glycoproteins and cell surface glycoproteins for a signal peptide and processing through the golgi for expression, so that selection for a molecule that illustrates an improvement in expression levels on the cell surface will also select for a molecule that illustrates an improvement in levels of secreted protein.

#### **EXAMPLE 45**

##### **CHARACTERIZATION OF G28-1 MABS AND SCFVS**

Ability of G28-1 mAbs and scFvs to induce apoptosis was measured by binding Annexin V, using the methods described in Example 3. The results in Figure 71 demonstrate that the Annexin V binding of G28-1 antibodies and scFv is increased when treated together with 2H7 antibodies and scFv constructs.

#### **EXAMPLE 46**

##### **CONSTRUCTION OF FC2-2 SCFV CONSTRUCTS**

Contrstruction of the FC2-2 (anti-CD16) scFv was performed using total RNA isolated from the FC2-2 hybridoma and cloned using methods described in Example

35. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_. The specific primers for the secondary PCR reaction are listed bellow. The following are primers for the light chain variable region:

5 5' primer with HindIII site with no leader: 5'-GTTGTTAAGCTTGCCGCCATGGATTACAGGCCCAGGTTCTT-3' (SEQ ID NO \_\_)

5' primer with SalI site and leader: 5'-GTTGTTGTCGACATTGTGATGTCACAGTCTCCATCCTCCCTA-3' (SEQ ID NO \_\_)

3' primer:

10 5'-TCAGTGCTGATCATGAGGAGACGGTGACTGAGGTTTCCTT-3' (SEQ ID NO \_\_)

The following are primers for the heavy chain variable region:

5' primer: 5'-

TCGGGCGGTGGTGGGTGCGGTGGCGGCGGATCGTCACAGGTGCAGTTGAAGGAGTCAGGA-3' (SEQ ID NO \_\_)

15 3' primer: 5'-

ACCCGACCCACCACCGCCCGAGCCACCGCCACCTTTTATTTCCAGCTTG  
GTGCCACCTCCGAA-3' (SEQ ID NO \_\_)

20 A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the FC2-2 scFv by site-directed mutagenesis according to the methods described in Example 33. The scFv was attached to the (SSS-S) H WCH2 WCH3 IgG tail according to methods described in Example 33. The mutant is designated FC2-2 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

25

#### EXAMPLE 47

##### CONSTRUCTION OF 5B9 scFv CONSTRUCTS

Contrstruction of the 5B9 (anti-CD137) scFv was performed using total RNA isolated from the 5B9 hybridoma and cloned using methods described in Example 35. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide

sequence is provided in SEQ ID NO:\_\_\_\_. The specific primers for the secondary PCR reaction are listed bellow. The following are primers for the light chain variable region:

5' primer with HindIII site with no leader: 5'-GTTGTTAAGCTTGCCGCCATGAGGTTCTCTGCTCAGCTTCTG-3' (SEQ ID NO\_\_)

5 5' primer with SalI site and leader: 5'-GTTGTTGTCGACATTTGTGATGACGCAGGCTGCATTCTCCAATT-3' (SEQ ID NO\_\_)

3' primer: 5'-TCAGTGCTGATCAGAGGAGGACGGTGACTGAGGTTTCCTTG-3' (SEQ ID NO\_\_)

The following are primers for the heavy chain variable region:

10 5' primer: 5'-  
CGGGCGGTGGTGGGTCGGGTGGCGGCGGATCGTCACAGGTGCAGCTGA  
AGCAGTCAGGA-3' (SEQ ID NO\_\_)

3' primer: 5'-  
CCCGACCCACCACCGCCCGAGCCACCGCCACCCTTCAGCTCCAGCTTG

15 GTGCCAGCACC-3' (SEQ ID NO\_\_)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the 5B9 scFv by site-directed mutagenesis and attached to (SSS-S) H WCH2 WCH3 according to the methods described in Example 33. This construct was designated 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3. The  
20 polynucleotide sequence is provided in SEQ ID NO:\_\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_.

#### EXAMPLE 48

##### CONSTRUCTION OF UCHL1 scFv CONSTRUCTS

Contrstruction of the UCHL1 (anti-CD45RO) scFv was performed using total  
25 RNA isolated from the UCHL1 hybridoma and cloned using methods described in Example 35 The polynucleotide sequence is provided in SEQ ID NO:\_\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_. The following are primers for the light chain variable region:

5' primer with HindIII site: 5'-

GTTGTTAAGCTTGCCGCCATGAAGTTGCCTGTTAGGCTG

TTGGTGCTG-3' (SEQ ID NO\_\_)

3' primer with Sac site: 5'-

5 AGAGCTCCACCTCCTCCAGATCCACCACCGCCCGAGCCAC

CGCCATCTTTGATTTCAGCTTGGT-3' (SEQ ID NO\_\_)

The following are primers for the heavy chain variable region:

5' primer: 5'-

TTTCAGAGTAATCTGAGAGCTCCACCTCCTCCAGATCCACCACCGC

10 CCGA-3' (SEQ ID NO\_\_)

3' primer: 5'-TCAGTGCTGATCATGCAGAGAGACAGTGACCAGAGTCCC-3' (SEQ ID NO\_\_)

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the 5B9 scFv by site-directed mutagenesis and connected to a(SSS-S) HWCH2 WCH3 according to the methods described in Example 33. The mutant is designated UCHL1 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 49

#### 20 L6 VHL11S scFv (SSS-S) H WCH2 WCH3

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the L6 scFv (SSS-S) H WCH2 WCH3 (constructed according to methods described in Example 106) by site-directed mutagenesis according to the methods described in Example 33. The L6scFvIg (SSS-S) H WCH2 WCH3 pD18 plasmid was used as template. Positive clones were inserted into the pD18 plasmid containing (SSS-S) H WCH2 WCH3 according to methods described in Example 33. The mutant is designated L6 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

PCR primers are listed bellow:

5' Primer with PstI restriction site:5'-

ggcggatctctgcagatccagttggtgcagctctggacctgagtcgaagaagcct  
ggagag-3' (SEQ ID NO\_\_)

5 3'Primer: 5'-ggacagtgggagtgacc-3' (SEQ ID NO\_\_)

#### EXAMPLE 50

##### CONSTRUCTION OF HD37 scFv VHL11S CONSTRUCT

A change from leucine to serine at position 11 in the heavy chain variable region (Kabat numbering) was introduced in into the HD37 scFv by site-directed  
10 mutagenesis according to the methods described in Example 33. The HD37 scFv (SSS-S)H WCH2 WCH3 pD18 plasmid was used as a template. Positive clones were inserted into the pD18 plasmid containing (SSS-S) H WCH2 WCH3 according to methods described in Example 33. The mutant is designated HD37 scFv VH L11S (SSS-S) H WCH2 WCH3. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide  
15 sequence is provided in SEQ ID NO:\_\_. PCR primers are listed bellow:  
5' primer: 5'-caggttcagctgcagcagctctgggctgagtcggtgaggcctgg-3' (SEQ ID NO\_\_)  
3' primer: 5'-ggaggattcgtctgcagtcagagtggc-3' (SEQ ID NO\_\_)

#### EXAMPLE 51

##### 2H7 scFv VHL11S CONSTRUCTS

20 Additional 2H7 VHL11S constructs were made with different connecting regions. The pD18 2H7 scFv VHL11S (SSS-S) H WCH2 WCH3 vector was digested with BclI and XbaI to remove the connecting region, CH2 and CH3. Theses were replaced with each different connecting region, CH2 and CH3 according to the methods described in Example 13. The new constructs were designated: 2H7scFv VHL11S (CSS-S) H WH2  
25 WH3 (SEQ ID NO\_\_), 2H7scFv VHL11S (CSC-S) H WH2 WH3 (SEQ ID NO\_\_). 2H7 scFv VHL11S was also attached to an IgA connecting region, CH2, CH3 and an IgE CH2, CH3, CH4. The pD18 2H7 scFv VHL11S (SSS-S) H WCH2 WCH3 plasmid was digested using methods above to remove the connecting region CH2, and CH3. The IgA regions were inserted using methods described in Example 13. The construct was designated 2H7

scFv VHL11S IgAH IgACH2 T4CH3 (SEQ ID NO \_\_\_\_). The IgE CH2 CH3 CH4 region was inserted into the digested pD18 vector above using methods described in Example 39. The construct was designated 2H7 scFv VHL11S IgECH2 CH3 CH4 (SEQ ID NO \_\_\_\_).

#### EXAMPLE 52

##### 5                    **2H7 scFv VH L11S (CSC-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a human IgG1 connecting region, CH2 and CH3 region, where in the second  
10    cysteine and the proline in the connecting region have been changed to serines (SSS-S) as described in Example 32. . The polynucleotide sequence is provided in SEQ ID NO: \_\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_\_.

#### EXAMPLE 53

##### 15                    **2H7 scFv VH L11S IgE CH2 CH3 CH4**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. . The polynucleotide sequence is provided in SEQ ID NO: \_\_\_\_, and the encoded  
20    polypeptide sequence is provided in SEQ ID NO: \_\_\_\_.

#### EXAMPLE 54

##### 25                    **2H7 scFv VH L11S mIgE CH2 CH3 CH4**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the  
25    leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mouse IgE constant region containing CH2, CH3 and CH4 as described in Example 39. . The polynucleotide sequence is provided in SEQ ID NO: \_\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_\_.



## EXAMPLE 55

### 2H7 scFv VH L11S mIgAH WIGACH2 T4CH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

## EXAMPLE 56

### 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322S mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a serine using overlapping PCR assay. An (SSS-S) H WCH2 WCH3 IgG1 template in the pD18 vector was used for PCR amplification, to create (SSS-S) H derivatives containing these CH2 mutations. PCR reactions used a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles to complete the reactions. This IgG1 derivative was constructed by using sequential PCR reactions with overlapping oligonucleotides in the primary and secondary reactions. The primary amplification primers introduced the mutation(s), but deleted one end of the Fc domain. Secondary reaction primers reattached these ends using overlapping primers. The first overlapping primer was added at the beginning of the PCR, the reactions allowed to proceed for 12 cycles, paused and then the

second overlapping primer added to the reactions followed by 25 more cycles to complete the overlap extension PCR reactions.

Primers for the first PCR reaction:

5' Primer: 5'-ggagatggtttctcgatgggggctgggaggccttggagaccgagcactgtactcc-3' (SEQ ID NO )

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO )

PCR products were cloned into TOPO vector and sequenced for verification.

Positive vectors were used as templates for the second overlapping PCR reaction.

5' primer: 5'-ccgtctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO )

5'primer overlapping primer: 5'-

ccccaccgtccccagcacctgaactcctggggggatcgtcagtcttctcttcccc

caaaacc-3' (SEQ ID NO )

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO )

15 PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 57

##### **2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3**

20 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to  
25 methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322S mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a serine using overlapping PCR assay. region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The mutation in the CH2 region was added by overlapping PCR reaction essentially according to

Example 57, with (CSS-S) H WCH2 WHC3 IgG1 pD18 vector as a template in the first PCR reaction and different primers in the second PCR reaction, which are listed below.

5'primer: 5'- ccgtctctgatcaggaccccaaatcttctgtacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO\_\_)

5 5'overlapping primer: 5'-

tccccaccgtcccccagcacctgaactcctggggggatcgctcagtcttctcttccccccaaaacc-3'

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO )

PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide

10 sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 58

##### 2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the  
15 leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG1 CH2 region and a wild type IgG1 CH3 region. The mutation P331S mutation in the CH2 region, where the proline at residue 331  
20 has been changed to a serine, was incorporated using a single PCR reaction, using a (SSS-S) H WCH2 WCH3 pD18 template and a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles. The specific primers for reaction are listed below.

5' primer: 5'ccgtctctgatcaggagcccaaatcttctgtacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO )

25 3' Primers: 5'-  
gcagggtgtacacctgtggttctcggggctgccctttggctttggagatggtttctcgatggaggctgggagg-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 59

##### **2H7 scFv VH L11S (CSS-S) H P331S CH2 WCH3**

This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The mutation P331S mutation in the CH2 region, where the proline at residue 331 has been changed to a serine, was incorporated using a single PCR reaction, using a (CSS-S) H WCH2 WCH3 pD18 template and a cycling profile of 94C, 30 sec; 55C, 30 sec; 72C, 30 sec, for 37 cycles.

10 The specific primers for reaction are listed below.

5' primer: 5'-ccgtctctgatcaggacccccaaatcttgacaaaactcacatccccaccgtccccagc-3' (SEQ ID NO )

3' Primer: 5'-gcagggtgtacacctgtggttctcggggctgccctttggctttggagatggtttctcgatggaggctgggagg-3' (SEQ ID NO )

15 The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### EXAMPLE 60

##### **2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3**

20 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The T256N mutation in the CH2 region, the threonine at residue 256 has been changed to an asparagine, using the overlapping PCR methods described in Example 56. The specific primers are listed below.

Primers for the first PCR reaction:

5' primer: 5'ttcctcttccccccaaaaccaaggacaccctcatgatctcccggaaacctgaggtcac-3' (SEQ ID NO )

3' primer: 5'-ggacagtgggagtgacc-3' (SEQ ID NO )

PCR product cloned into TOPO vector and sequenced. This product was used as the template in the second PCR reaction. Primers for the second PCR reaction:

5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO )

5'overlapping primer: 5'-

tccccaccgtccccagcacctgaactcctggggggatcgtagcttctcttccccccaaaacc-3' (SEQ ID NO )

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 61

##### **2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The RTPE/QNAK mutation in the CH2 region, where residues 255-258 have been mutated from arginine, threonine, proline, glutamic acid to glutamine, asparagines, alanine and lysine, respectively, using the overlapping PCR reactions described in Example 56. The specific primers are listed below.

PCR primers for the first PCR reaction:

5' primer: 5'-ttctcttccccccaaaaccaaggacaccctcatgatctcccagaacgctaagggtcacatgc-3' (SEQ ID NO \_\_)

3' primer: 5'-ggacagtgggagtgacc-3' (SEQ ID NO )

The PCR product was cloned into TOPO vector, sequenced and used as a template for the second PCR reaction. The primers for the second PCR reaction:

5 primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO )

5 5'overlapping primer:5'-

tccccaccgtccccagcacctgaactctgggggatcgtcagtcttcttccccccaaaacc-3' (SEQ ID NO )

3' primer: 5'-caggaaacagctatgac-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

10

#### EXAMPLE 62

##### **2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The K290Q mutation in the CH2 region, where the Lysine at residue 290 has been changed to a Glutamine, using a single PCR reaction according to the methods described in Example 58. The specific primers used in this reaction are listed below.

15 5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO )

20 3' primer: 5'-gctcccgcggtgtgtcttggc-3' (SEQ ID NO )

25 PCR products were cloned into TOPO and sequenced. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

#### EXAMPLE 63

##### **2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. The connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The A339P mutation in the CH2 region, where the alanine at residue 339 has been changed to a proline, using a single PCR reaction according to the methods described in Example 58. The specific primers used in this reaction are listed below.

5' primer: 5'ccgtctctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagc-3' (SEQ ID NO )

3' Primer: 5'-ggaggtgggcagggtgtacacctgtggttctcggggctgccctttgggttggagatgg-3' (SEQ ID NO )

PCR products were cloned into TOPO and sequenced. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 64

##### G28-1 scFv (SSS-S) H WCH2 WCH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region made according to methods described in Example 35. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. This construct has previously been referred to as G28-1- MHW TG1C and G28-1 scFv Ig, both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 65

### **G28-1 scFv IgAH WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region made according to methods described in Example 35. This binding region is connected to a human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5. This construct has previously been referred to as: G28-1-IgAHWTG1C. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

### **EXAMPLE 66**

#### **G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3**

10 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5.

15 This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

### **EXAMPLE 67**

#### **G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3**

20 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according

25 to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.



#### EXAMPLE 68

##### **G28-1 scFv VH L11S (CSC-S) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the second cysteine and the proline has been changed to serine (CSC-S), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 69

##### **G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the first and second cysteines have been changed to serines (SSC-P), according to methods described in Example 13. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 70

##### **CTLA4 (SSS-S) H P238SCH2 WCH3**

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced using a PCR assay. PCR reactions were performed using random primed cDNA prepared from human tonsil B cell RNA. PCR

amplifications used an amplification profile of 94C 4 min; [94C 1 min; 55C 1 min; 72C 1min; for 30 cycles followed by a final extension step for 6 minutes at 72C. PCR fragments were TOPO cloned and clones with EcoRI inserts of approximately 800 bp were sequenced as described in Example 1. The primers used for the PCR are listed below:

5 5' primer: 5'-

gtgttgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtccccagcacctgaactcctgggtgg  
accgtcagtcttcc-3' (SEQ ID NO )

3' primer: 5'gtgtttctagattatcatttaccggagacag-3' (SEQ ID NO )

This construct has previously been referred to as CTLA-4 IgG MTH (SSS)  
10 MTCH2WTCH3, which has the same sequence as the above construct. The polynucleotide  
sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided  
in SEQ ID NO:\_\_.

#### EXAMPLE 71

##### **CTLA4 (CCC-P) WH WCH2 WCH3**

15 This construct has a CTLA-4 binding region as described in Example 14.  
This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as  
described in Example 1. This connecting region is connected to a wild type human IgG1  
CH2 and CH3 region as described in Example 1. This construct has previously been  
referred to as CTLA-4 IgG WTH (CCC) WTCH2CH3, which has the same sequence as the  
20 above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the  
encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 72

##### **FC2-2 scFv (SSS-S) H WCH2 WCH3**

25 This construct has a FC2-2 (anti-CD16) single chain Fv made according to  
methods described in Example 46. This binding region is connected to a mutated human  
IgG1 connecting region where all of the cysteines and one proline have been changed to  
serines (SSS-S) according to methods described in Example 5. This connecting region is  
connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The

polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### **EXAMPLE 73**

##### **FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3**

5                   This construct has a FC2-2 (anti-CD16) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 46. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting  
10                   region is connected to a wild type human IgG1 CH2 and CH3 region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### **EXAMPLE 74**

##### **UCHL-1 scFv (SSS-S) H WCH2 WCH3**

15                   This construct has a UCHL-1 (anti-CD45RO) single chain Fv made according to methods described in Example 48. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 region as described in  
20                   Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### **EXAMPLE 75**

##### **UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3**

25                   This construct has a UCHL-1 (anti-CD45RO) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 48. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting

region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 76

##### 5 5B9 scFv (SSS-S) H WCH2 WCH3

This construct has a 5B9 (anti-CD137) single chain Fv made according to methods described in Example 47. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is  
10 connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. This construct has previously been referred to as 5B9 scFv IgG MTH (SSS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### 15 EXAMPLE 77

##### 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3

This construct has a 5B9 (anti-CD137) single chain Fv with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 47. This binding region is connected to a  
20 mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type human IgG1 CH2 and CH3 constant region as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### 25 EXAMPLE 78

##### 2H7 scFv (SSS-S) H WCH2 WCH3

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is connected to a wild type IgG1 CH2 and CH3 constant region as described in Example 1. This construct has previously been referred to as 2H7-MHWTG1C, CytoxB-(MHWTG1C) – Ig, anti-CD20 scFv IgG MTH (SSS) WTCH2CH3, CytoxB-MHWTG1C, 2H7 scFv-human IgG1 wild type hinge-CH2-CH3, and 2H7 scFv IgG MTH (SSS) WTCH2CH3, which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### EXAMPLE 79

##### **2H7 scFv (SSS-S) H P238SCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This construct has previously been referred to as 2H7 scFv IgG MTH (SSS) MTCH2WTCH3, anti-CD20 scFv IgG MTH (SSS) MTCH2CH3, and CytoxB-MHMG1C which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### EXAMPLE 80

##### **2H7 scFv IgAH WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a human IgA connecting region and wild type human IgG1 CH2 and CH3 constant regions as described in Example

5. This construct has previously been referred to as 2H7 scFv IgAH IgG WTCH2CH3, 2H7 scFv IgA hinge-IgG1 CH2-CH3, and CytosB-IgAHWTHG1C, which all have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

5

#### EXAMPLE 81

##### **2H7 scFv IgAH WIGACH2 T4CH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This construct has previously been referred to as 2H7 scFv IgAH IgAT4, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

15

#### EXAMPLE 82

##### **2H7 scFv IgAH WIGACH2 WCH3 + JCHAIN**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. This constant region is attached to a J-chain region as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

25

#### EXAMPLE 83

##### **2H7 scFv (CCC-P) WH WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached

to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv Ig WTH (CCC) WTCH2CH3, 2H7 scFv IgG WTH WTCH2CH3, and 2H7 scFv-Ig, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 84

##### **2H7 scFv (SSS-S) H WCH2 F405YCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405Y mutation, where the phenylalanine at residue 405 has been changed to a tyrosine, was introduced according to methods described in Example 21. This construct has previously been referred to as 2H7 scFv MTH WTCH2 MTCH3 Y405, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 85

##### **2H7 scFv (SSS-S) H WCH2 F405ACH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405A mutation, where the phenylalanine at residue 405 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as 2H7 scFv MTH WTCH2 MTCH3 A405, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 86

##### **2H7 scFv (SSS-S) H WCH2 Y407ACH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The Y407A mutation, where the tyrosine at residue 407 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as scFv MTH WTCH2 MTCH3 A407, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 87

##### **2H7 scFv (SSS-S) HWCH2 F405A, Y407ACH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5. This connecting region is attached to a wild type human IgG1 CH2 and a mutated human IgG1 CH3 region. The F405A and Y407A mutation, where the phenylalanine at residue 405 has been changed to an alanine and the tyrosine at residue 407 has been changed to an alanine, was introduced according to methods described in Example 21. This construct has previously been referred to as scFv MTH WTCH2 MTCH3 A405A407, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 88

##### **2H7 scFv (CSS-S) H WCH2 WCH3**



This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CSS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### **Example 89**

##### **2H7 scFv (SCS-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the first and third cysteines have been changed to serines and the proline has been changed to serine (SCS-S), according to methods described in Example13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv IgG MTH (SCS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### **EXAMPLE 90**

##### **2H7 scFv (SSC-P) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the first and second cysteines have been changed to serines (SSC-P), according to methods described in Example13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (SSC) WTCH2CH3, which has

the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 91

##### **2H7 scFv (CSC-S) H WCH2 WCH3**

5 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where the second cysteine and the proline has been changed to serine (CSC-S), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example

10 1. This construct has previously been referred to as 2H7 scFv MTH (CSC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 92

##### **2H7 scFv (CCS-P) H WCH2 WCH3**

15 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where third cysteine has been changed to a serine (CCS-P), according to methods described in Example 22. This connecting region is attached to wild type human

20 IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (CCS) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 93

##### **2H7 scFv (SCC-P) H WCH2 WCH3**

25 This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a mutant human IgG1 connecting region, where first cysteine has been changed to a serine (SCC-P), according to

methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv MTH (SCC) WTCH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 94

##### **2H7 scFv VH L11S (SSS-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3 and 2H7 scFv VHSER11 WTH WTCH2CH3, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 95

##### **2H7 scFv VH L11S (CSS-S) H WCH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 33. This binding region is attached to a mutant human IgG1 connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

### **Example 96**

#### **G28-1 scFv VH L11S (SCS-S) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where the first and third cysteines have been changed to serines and the proline has been changed to serine (SCS-S), according to methods described in Example 13. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

### **EXAMPLE 97**

#### **G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where third cysteine has been changed to a serine (CCS-P), according to methods described in Example 22. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

### **EXAMPLE 98**

#### **G28-1 scFv VH L11S (SCC-P) H WCH2 WCH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a mutant human IgG1 connecting region, where first cysteine has been changed to a serine (SCC-P), according to methods described in Example 32. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in

Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 99

##### **G28-1 scFv VH L11S mIgE CH2 CH3 CH4**

5 This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a wild type mouse IgE CH2 CH3 and CH4 region using methods described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded  
10 polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 100

##### **G28-1 scFv VH L11S mIgAH WIGACH2 T4CH3**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the  
15 leucine has been changed to a serine as described in Example 35. This binding region is attached to a connecting region from mouse IgA as described in Example 39. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a 4 amino acid truncation at residues as described in Example 39. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and  
20 the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 101

##### **G28-1 scFv VH L11S hIgE CH2 CH3 CH4**

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the  
25 leucine has been changed to a serine as described in Example 35. This binding region is attached to a wild type human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

## EXAMPLE 102

### G28-1 scFv VH L11S HIGAH WIGACH2 T4CH3

This construct has a G28-1 (anti-CD37) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine as described in Example 35. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

## EXAMPLE 103

### HD37 scFv IgAH WCH2 WCH3

The HD37 scFv was cloned from the HD37 hybridoma using the Novagen – Ig family primer sets, TOPO cloning and sequencing and sewing PCR assay. For the initial PCR reactions prior to sewing, the TOPO clone templates HD37 VH C-1 and HD37 KVL B-9 were used at 1:100 with an amplification profile of 94C 30 sec; 55C, 30 sec; 72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile: 94C, 60 sec; 55C, 60 sec; 72C, 60 sec; for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors.

PCR of VL domain with native leader peptide and part of glyser linker:

5' primer: 5'-gttgtaagcttgccgccatggagacagacacactcctgctatgg-3' (SEQ ID NO )

3' primer: 5'gccacccgacccaccaccgcccagaccgccaccttgattccagcttggtgcctcc-3' (SEQ ID NO )

PCR of VL domain without leader peptide (SalI site) and part of glyser linker:

5' primer: 5'-gttggtgacattgtgctgacccaatctcca-3' (SEQ ID NO )

3' priemr: 5'-gccacccgacccaccaccgcccgagccaccgccaccttgattccagcttggtgcctcc-3' (SEQ ID NO )

PCR of VH domain with part of glyser linker and BclI site for fusion to -Ig tails.

5'primer: 5'-tcgggcggtggtgggtcgggtggcggcgatcgtcacaggttcagctgcagcagtctgg-3' (SEQ ID NO )

3' primer: 5'-tcagtgtgatcagaggagacgggtgactgaggttccttg-3' (SEQ ID NO )

This binding region is connected to a wild type human IgA connecting region and wild type human IgG CH2 and CH3 constant regions as described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as HD37 scFv-IgAHWTG1C and HD37- IgAHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 104

##### **HD37 scFv (SSS-S) H WCH2 WCH3**

This construct has a HD 37 single chain Fv as described in Example 103. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as HD37- MHWTG1C and HD37 scFv-IgMHWTG1C, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 105

##### **HD37 scFv VH L11S (SSS-S) H WCH2 WCH3**

This construct has a HD 37 single chain Fv with a mutation in the heavy chain variable region at amino acid residue 11, where leucine has been changed to serine according to the methods described in Example 50. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 106

##### 10 L6 scFv IgAH WCH2 WCH3

The L6 scFv was cloned from the L6 hybridoma (I Hellstrom) using the anchor-tailing method described in the Tissue Antigens Paper from 1996. The PCR profile was 94C, 1 min; 50C, 2 min; 72C, 2 min; for 35 cycles. Once consensus sequence was obtained for VL and VH regions from at least 4 TOPO clones, primers were ordered for PCR reactions prior to SEWING PCR reactions as follows: For the initial PCR reactions prior to sewing, the TOPO cloned templates L6VK and L6VH were used at 1:100 with an amplification profile of 94C 30 sec; 55C, 30 sec; 72C, 30 seconds for 25 cycles. To provide templates for the secondary SEWING PCR reactions, primary reaction products were gel purified, QIAQUICK purified, and the eluates diluted 50 fold. One microliter each VL and VH overlapping templates were added to PCR reactions with the following amplification profile: 94C, 60 sec; 55C, 60 sec; 72C, 60 sec; for 30 cycles. After two cycles, the machine was paused, and the flanking 5'VL and 3'VH primers were added to the reactions at 25 pmol each, and the PCR reactions resumed. PCR products were checked on a gel for the presence of an 750-800 bp fragment, and the reactions products QIAQUICK purified and digested with the appropriate restriction enzymes for insertion into pD18 Ig expression vectors.

PCR of VL domain with native leader peptide and part of glyser linker:

L6VLHindIII: 5'-gttgtaagcttgccgcatggatttcaagtcagatttcagcttc-3' (SEQ ID NO )



L6VLLK3: 5'-gccacccgacccaccaccgcccagccaccgcccaccagagagctctttcagctccagcttggt-3'  
(SEQ ID NO )

PCR of VL domain without leader peptide (Sall site) and part of glyser  
linker:

- 5 5' primer: 5'-gttggtgacattgttctctcccagctccagcaatcctgtctg-3' (SEQ ID NO )  
3' primer: 5'-gccacccgacccaccaccgcccagccaccgcccaccagagagctctttcagctccagcttggt-3' (SEQ  
ID NO )

PCR of VH domain with part of glyser linker and BclI site for fusion to -Ig  
tails.

- 10 5': 5'-tcgggcggtggtgggtcgggtggcgccgagatctctgcagatccagttggtgcagtct-3' (SEQ ID NO )  
3'Bcl: 5'-tcagtgtgatcagaggagactgtgagagtggcgcttg-3' (SEQ ID NO )

- This binding region is connected to a human IgA connecting region and wild  
type human IgG1 CH2 and CH3 constant regions as described in Example 5. This  
connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as  
15 described in Example 1. This construct has previously been referred to as L6 scFv-  
IgAHWTG1C, which HAS the same sequence as the above construct. The polynucleotide  
sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided  
in SEQ ID NO:\_\_.

#### EXAMPLE 107

##### 20 L6 scFv VHL11S (SSS-S) H WCH2 WCH3

- This construct has a L6 single chain Fv with a mutation in the heavy chain  
variable region at amino acid residue 11, where leucine has been changed to serine  
according to the methods described in Example 49. This binding region is connected to a  
mutated human IgG1 connecting region where all of the cysteines and one proline have been  
25 changed to serines (SSS-S) according to methods described in Example 5. This connecting  
region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in  
Example 1. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded  
polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 108

### **2H7 scFv-LLAMA IGG1**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG1 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

### **EXAMPLE 109**

### **2H7 scFv-LLAMA IGG2**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG2 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

### **EXAMPLE 110**

### **2H7 scFv-LLAMA IGG3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a llama IgG3 hinge, CH2 and CH3 regions according to the methods described in Example 10. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

### **EXAMPLE 111**

### **CD16 LOW (ED)(SSS-S) H P238SCH2 WCH3**

This construct has the extra cellular, CD16 low affinity allele binding domain as described in Example 43. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according

to methods described in Example 70. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### **EXAMPLE 112**

##### **CD16-9 HIGH (ED)(SSS-S) H P238SCH2 WCH3**

- 5           This construct has the extra cellular, CD16 high affinity allele binding domain as described in Example 43. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region.
- 10   The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### **Example 113**

##### **2e12 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

- 15           This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S
- 20   mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a human CD80 transmembrane and cytoplasmic tail region (hCD80 TM/CT). The hCD80 TM/CT was cloned using random primed cDNA derived from the BJAB cell line according to methods described in Example 12. This TM/CT region was attached to a Ig CH3 region with an
- 25   open reading frame (ORF). Open reading frame versions of scFvIg constructs of interest were created by replacement of the soluble versions of each –Ig tail with ORF (open reading frame versions) of these tails. PCR primers were designed for the existing clones of soluble –Ig tails which delete the stop codon and add one or more restriction sites to the 3' end of the new –Ig cassettes. The desired transmembrane and cytoplasmic tail sequences can then

be subcloned downstream of these new –Ig cassettes. Each construct utilized the existing available 5' BCLI oligonucleotide used in amplifying the soluble version of the tails for the PCR reactions. The 3' oligonucleotides replace the stop codon with out of frame restriction sites fused to the coding region for protein domains involved in regulation of apoptosis.

5                   The PCR amplifications were carried out with 25 pmol of each primer, standard PCR reagents, and varying volumes of either cloned domains or cDNA obtained from PBMC, spleen, or thymus RNA. The reactions used a cycling profile of 94C, 60sec; 55C, 60 sec; 72C, 2 min, for 35 cycles. The primers for the IgG ORF are listed below.

5' primer: 5'-gtttagatcaggagcccaaatcttctgacaaaactcacacatctccaccgtccccagcacctgaactcctggg

10 ggaccgtcagttctcc-3'(SEQ ID NO )

3' primer: 5'-gttggtttcgaaggatccgctttacccgggagcagggagaggctcttctgcgtgtagtg-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

15

#### EXAMPLE 114

##### **10A8 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

This construct has a 10A8 (anti-CD2152) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The polynucleotide sequence is provided in

20

25   SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 115

##### **40.2.220 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

This construct has a 40.2.220 (anti-CD40) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO: \_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_.

#### EXAMPLE 116

##### **2H7 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S), according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. This construct has previously been referred to as: The polynucleotide sequence is provided in SEQ ID NO: \_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_.

#### EXAMPLE 117

##### **G19-4 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. This CH3 region is attached to a hCD80 TM/CT

according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

#### EXAMPLE 118

##### **2E12 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT**

5           This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This  
10   CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. This construct has previously been referred to as 2e12 scFv IgG WTH WHTCH3CH2-CD80, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

15

#### EXAMPLE 119

##### **2E12 scFv IGAH IGACH2 T4CH3—hCD80TM/CT**

          This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA  
20   constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in example 13. This CH3 region is attached to a hCD80 TM/CT according to methods described in Example 113. The specific primers used to create an IgA ORF are listed below.

5'primer: 5'-gttggtgatcagccagttccctcaactccacctacc-3' (SEQ ID NO )

25   3' primer: 5'-gttggtttcgaaggatccgcgtccacctccgcatgacaacaga (SEQ ID NO )

          The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_\_\_.

#### EXAMPLE 120

### **2E12 scFv IgE CH2CH3CH4—hCD80TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. This CH4 region is attached to a hCD80 TM/CT essentially according to methods described in Example 113. The specific primers used to create an IgE ORF are listed below.

5' primer: 5'-gttgatgacacgtctgctccaggacttcacc-3' (SEQ ID NO )

3'primer: 5'-gtgttttcgaaggatccgctttaccagattacagacaccgctcgctg-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

### **EXAMPLE 121**

### **2E12 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a mouse FADD transmembrane and cytoplasmic tail region (mFADD TM/CT). This region is cloned using essentially the same methods described in Example 113. The domain was PCR amplified from randomly primed cDNA from mouse spleen RNA. The specific primers are listed below.

5' primer: 5'-gttgatgacacgtctgctccaggacttcacc-3' (SEQ ID NO )

3' primer: 5'-gtgttatcgatctcgagtcagggtgtttctgaggaagacacagt-3' (SEQ ID NO )

The specific primers used to create a mouse IgG ORF are listed below.

5'primer: 5'-gtttagatctggagcccagagggcccacaatcaagccctctcctcaagcaaaagccca-3' (SEQ ID NO )

3'primer: 5'-gtgttttcgaaggatccgctttacccggagtcgggagaag-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### EXAMPLE 122

##### 2E12 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT

5 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The  
10 CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### EXAMPLE 123

##### 2E12 scFv (SSS-S)H WCH2 WCH3—mCASP3-TM/CT

15 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The  
20 CH3 region is attached to a mouse casp3 transmembrane and cytoplasmic tail region according to methods described in Examples 113 and 121. The specific primers used to isolate the mcasp3 TM/CT region are listed below:

5' primer: 5'-gttgttgatccttcgaacatggagaacaacaaacctcagtgattca-3' (SEQ ID NO )

3' primer: 5'-gttgttatcgatctcgagctagtgtataaaagtacagttcttcgt-3' (SEQ ID NO )

25 The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

#### EXAMPLE 124

##### 2E12 scFv (SSS-S)H P238SCH2 WCH3—mCASP3-TM/CT



This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is connected to a mcasp3 TM/CT region according to methods described in Examples 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 125

##### **2E12 scFv (SSS-S)H WCH2 WCH3—MCASP8--TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a mouse casp8 transmembrane and cytoplasmic tail region (mcasp8 TM/CT) essentially according to methods described in Example 113 and 121. The specific primers used to clone the mcasp8 TM/CT region are listed below.

5' primer: 5'-gtgtttcgaacatggattccagagtgctcttatgctattgctg-3' (SEQ ID NO )

3' primer: 5'-gtgttatcgatctcgagtcattagggagggagaagaagagcttcttcg-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 126

##### **2E12 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines

(SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp8 TM/CT region according to methods described in Examples 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 127

##### 2E12 scFv (SSS-S)H WCH2 WCH3—HCASP3--TM/CT

10 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The  
15 CH3 region was attached to a human casp3 transmembrane and cytoplasmic tail region (hcasp3 TM/CT) essentially according to methods describe in Examples 113. The specific primers used to clone the hcasp3 TM/CT region are listed below.

5' Primer: 5'-gttgtggtatccttcgaacatggagaacactgaaaactcagtggat-3' (SEQ ID NO )

3' Primer: 5'-gttgttatcgatctcgagttagtgataaaaatagagttctttgtgag-3' (SEQ ID NO )

20 The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 128

##### 2E12 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT

25 This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according

to methods described in Example 70. The CH3 region is attached to a hcasp3 TM/CT region according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

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#### EXAMPLE 129

##### **2E12 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a human casp8 transmembrane and cytoplasmic tail region (hcasp8 TM/CT) according to methods described in Example 133. The specific primers used to clone the hcasp8 TM/CT region are listed below.

10 5' Primer: 5'-gttgtggtatccttcgaacatggacttcagcagaaatctttatgat-3' (SEQ ID NO )

3' Primer: 5'-gttgttatcgatgcagctcaatcagaagggaagacaagttttttct-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 130

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##### **2E12 scFv (SSS-S)H P238SCH2 WCH3—HCASP8-TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is

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provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 131

##### **1D8 scFv (SSS-S)H P238SCH2 WCH3—hCD80TM/CT**

5           This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S  
10       mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### 15           EXAMPLE 132

##### **1D8 scFv (SSS-S)H WCH2 WCH3—hCD80TM/CT**

          This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines  
20       (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hCD80 TM/CT region according to methods described in Example 113. This construct has previously been referred to as 1D8 scFv IgG WTH WTCH2CH3-CD80, which has the same sequence as the above construct. The  
25       polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 133

##### **1D8 scFv—mIgAH WIGA CH2 T4CH3—hCD80TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 39. The CH3 region can be attached to a hCD80 TM/CT region according to methods described in Examples 113 using primers that create an IgA ORF.

#### **EXAMPLE 134**

##### **1D8 scFv IgE CH2CH3CH4 -hCD80TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is attached to a human IgE constant region containing CH2, CH3 and CH4 as described in Example 38. The CH4 region is attached to a hCD80 TM/CT according to methods described in Examples 113 and 120. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### **EXAMPLE 135**

##### **1D8 scFv (SSS-S)H P238SCH2 WCH3—mFADD-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### **EXAMPLE 136**

**1D8 scFv (SSS-S)H WCH2 WCH3—mFADD-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mFADD TM/TM region according to methods described in Example 113 and 121. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_\_.

**EXAMPLE 137**

**1D8 scFv (SSS-S)H WCH2 WCH3—mcasp3-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_\_.

**EXAMPLE 138**

**1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP3-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp3 TM/TM region according to methods described in Example 113, 121 and 123. The polynucleotide

sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 139

##### **1D8 scFv (SSS-S)H WCH2 WCH3—MCASP8--TM/CT**

5           This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The  
10 CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 140

##### **1D8 scFv (SSS-S)H P238SCH2 WCH3—MCASP8-TM/CT**

15           This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S  
20 mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region was attached to a mcasp8 TM/TM region according to methods described in Example 113, 121 and 125. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

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#### EXAMPLE 141

##### **1D8 scFv (SSS-S)H WCH2 WCH3—HCASP3--TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1

connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 142

##### **1D8 scFv (SSS-S)H P238SCH2 WCH3—HCASP3-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp3 TM/CT according to methods described in Examples 113 and 127. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 143

##### **1D8 scFv (SSS-S)H WCH2 WCH3—HCASP8--TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.



#### EXAMPLE 144

##### **1D8 scFv (SSS-s)H P238SCH2 WCH3—HCASP8-TM/CT**

This construct has a 1D8 (anti-4-1BB) single chain Fv binding region described in Example 25. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This hinge region is attached to a mutated human IgG1 CH2 region and a wild type human IgG1 CH3 region. The P238S mutation, where a proline at residue 238 was changed to a serine, was introduced according to methods described in Example 70. The CH3 region is attached to a hcasp8 TM/CT according to methods described in Examples 113 and 129. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 145

##### **L6 scFv (SSS-S) H WCH2 WCH3**

This construct has a L6 scFv binding domain as described in Example 105. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been referred to as L6 scFv-IgMHWGTG1C, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 146

##### **2H7 scFv CD154 (L2)**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region has been attached to CD154 extracellular domain according to methods described in Example 4. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

#### EXAMPLE 147

##### **2H7 scFv CD154 (S4)**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region has been attached to CD154 extracellular domain according to methods described in Example 4, such that methods of attachment resulted in a truncated version compared to the construct describe in Example 146. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 148

##### 10 **CTLA4 IgAH IgACH2CH3**

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. This constant region is attached to a J-chain region as described in Example 13. This construct has previously been referred to as CTLA-4 IgAH IgACH2CH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 149

##### 20 **CTLA4 IgAH IgACH2 T4CH3**

This construct has the extra cellular CTLA-4 binding region as described in Example 14. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This construct has previously been referred to as CTLA-4 IgAH IgA-T4, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 150

### **2H7 scFv IgAH IgACH2CH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a wild type human IgA connecting region as described in Example 5. This connecting region is attached to a wild type human IgA CH2 and CH3 constant region according to methods described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

### **EXAMPLE 151**

### **2H7 scFv IgAH IgAHCH2 T18CH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 18 amino acid residues prior to the 3' stop codon as described in Example 13. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

### **EXAMPLE 152**

### **2H7-40.2.220 scFv (SSS-S) H WCH2 WCH3**

A bispecific fusion protein was constructed between 2H7 scFv (anti-CD20) and 40.2.220 (anti-CD40), which both target B cell receptors. The 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 construct in the expression vector pD18 was passaged through dam<sup>-</sup> bacteria in order to permit cleavage at the BclI site. Cleaved plasmid was treated with alkaline phosphatase prior to ligation to a BclI cut linker-CD40 scFv fragment. This fragment was synthesized from the existing 40.2.220 scFv by successive PCR reactions with overlapping primers. The linker attached is a previously patented (BMS patent issued) helical type linker with a high number of lysine and glutamic acid residues. The scFv for CD40 was PCR amplified without the leader peptide as a Sall-BclI fragment, but with the hinge type linker substituted at the amino terminus as a BclI-Sall fragment, to mediate insertion of the linker-scFv cassette as a BclI fragment between the scFv and -Ig tail

included in an existing scFvIg construct for CD20 (2H7 scFv hIgG1 constructs). The 3' end was similar to the other scFv VH molecules with an out of frame BclI site fused to the VTVSS type sequence at the end of the VH domain.

PCR oligos:

5 40.2.220 scFv:

5' primer--40.2.220S5: 5'-gttggtgacgacattgttctgactcagtcctccagccaccctgtc-3' (SEQ ID NO )

3' primer--40.2.220Bcl3: 5'-gttggtgatcagagacagtgcaccagtgtcccttgg-3' (SEQ ID NO )

Linker Primers:

10 BclI-SalI fragment created by annealing complementary oligonucleotides. This fragment is then ligated into BclI digested vector along with a SalI-BclI scFv to create the (linker-scFv) BclI fragment desired for shuttling.

5' primer: 5'-gatcaatccaactctgaagaagcaaagaagaggaggccaaaaaggaggaagccaagaaatctaacagcg-3' (SEQ ID NO )

15 3' primer: 5'-tcgacgctgtagatttcttgcttcctccttttggcctcctcttcttcttcttcagagttggatt-3' (SEQ ID NO )

This BclI fragment was then ligated downstream of the 2H7 scFv in pD18-Ig. Transformants were screened for the presence of a 2.4 kb HindIII-Xba insert and positive clones sequenced prior to further studies. COS cell transient transfections were performed with this construct and culture supernatants screened for the presence of protein of the predicted size and for binding to CD20 and to CD40 transfected CHO cells.

20 New bispecific constructs can be created by designing hinge type linkers which incorporate one or preferably two restriction sites at either end of the linker, facilitating asymmetric digests and transfer of (linker-scFv) or (scFv-linker) cassettes between different constructs. These constructs will also incorporate the VH L11S and other V region substitutions, which presumably facilitate proper folding and result in increased expression of the molecules in which they are inserted.

25 This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This construct has previously been

referred to as anti-CD20-anti-CD40 scFv IgG MTH (SSS) MTCH2WTCH3, which has the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

5

#### EXAMPLE 153

##### **2H7 scFv IgAH IgACH2 T4CH3-hCD80 TM/CT**

This construct has a 2H7 (anti-CD20) single chain Fv binding region as described in Example 1. This binding region is attached to a connecting region from human IgA as described in Example 5. This connecting region is attached to a human IgA constant region consisting of a wild type CH2 region and a mutated CH3 region where there is a truncation of 4 amino acid residues prior to the 3' stop codon as described in Example 13. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113 and 119. This construct has previously been referred to as 2H7 scFv IgA hinge IgA-T4-CD80 and 2H7 scFv IgAH IgA-T4-CD80, which both have the same sequence as the above construct. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

10

15

#### EXAMPLE 154

##### **G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD80 TM/CT**

This construct has a G19 (anti-CD3) single chain Fv binding region described in Example 29. This binding region is attached to a wild type human IgG1 connecting region (CCC-P) as described in Example 1. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_.

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25

#### EXAMPLE 155

##### **2e12 scFv (CCC-P) WH WCH2 WCH3-hCD80 TM/CT**

This construct has a 2e12 (anti-CD28) single chain Fv binding region described in Example 12. This binding region is attached to a wild type human IgG1

connecting region (CCC-P) as described in Example 1. This connecting region is attached to wild type human IgG1 CH2 and CH3 constant regions as described in Example 1. This CH3 region is attached to a hCD80 TM/CT according to the methods described in Examples 113. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 156

##### **2H7 VHL11S scFv (SSS-S) IgECH3CH4**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine. as described in Example 33. This binding region is connected to a mutated human IgG1 connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5. This connecting region is attached to human IgE CH3 and CH4 constant region. This truncated constant region was created according to the methods described in Example 38. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 157

##### **IGD HINGE**

An alternative hinge region can be isolated from human IgD immunoglobulin hinge region by using PCR assay to isolate the desired region. The PCR reaction is the same used in Example 1. This hinge was truncated by 6 amino acid residues at the 3' end. The primers used in this PCR reaction are listed below.

5'' Primer: 5'-GTGGATCCAGGTTCGAAGTCTCCAAAGGCACAGGCC-3' (SEQ ID NO )  
3' primer: 5'-GTTGTCGACTGCACCGGTCTTTGTCTCTCTCTCTTC-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_\_.

#### EXAMPLE 158

### **hCD28 TM/CT**

For some of the cell surface ORF constructs, the transmembrane domain of CD80 was substituted with the transmembrane domain of human CD28 because it forms a dimer on the cell surface rather than a monomer as the CD80 does. Several of the molecules which drive the apoptotic program require oligomerization/trimerization to form a signaling complex; therefore, it is important to be able to control initiation of signaling by controlling the degree of oligomerization of these recombinant receptors on the cell surface.

The primers used in PCR amplification of the CD28 tail are given below:

5' Primer: 5'-gttgtgcatccttcgaacccctttgggtgctggtggtggtgga-3' (SEQ ID NO )

10 3' primer: 5'-gttggtatcgatctcgagtcaggagcgataggctgcgaagtc-3' (SEQ ID NO )

The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

### **EXAMPLE 159**

#### **2H7 scFv VH L11S (SSS-S) H K322L CH2 WCH3**

15 This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine. as described in Example 33. This binding region is connected to a mutated human IgG connecting region where all of the cysteines and one proline have been changed to serines (SSS-S) according to methods described in Example 5.

20 The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at a residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, but with different primers for the first PCR reaction, which are listed below.

5' primer: 5'ttctcttcccccaaaaccaagacaccctcatgatctcccggaaccctgaggtcac-3' (SEQ ID NO )

25 )

3' primer: 5'-ggacagtgggagtggcacc-3' (SEQ ID NO )

PCR product was cloned into TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO:\_\_, and the encoded polypeptide sequence is provided in SEQ ID NO:\_\_.

**EXAMPLE 160**  
**2H7 scFv VH L11S (CSS-S) H K322L CH2 WCH3**

This construct has a 2H7 (anti-CD20) single chain Fv binding region with a point mutation at amino acid residue 11 in the heavy chain variable region, where the leucine has been changed to a serine, as described in Example 33. This binding region is attached to a mutant IgG connecting region, where the second and third cysteines have been changed to serines and the proline has been changed to serine (CSS-S), according to methods described in Example 13. The connecting region is attached to a mutated IgG CH2 region and a wild type IgG CH3 region. The K322L mutation in the CH2 region is at residue 322, where a Lysine has been changed to a leucine using overlapping PCR described in Example 56, using primers from Example 159 in the first PCR reaction and primers from Example 57 for the second PCR reaction.

PCR products were cloned into the TOPO vector and sequenced. The polynucleotide sequence is provided in SEQ ID NO: \_\_, and the encoded polypeptide sequence is provided in SEQ ID NO: \_\_\_\_.

Additional representative constructs or sequences within various of examples of the present invention are as follows:

NT

**HuIgG1 wild type hinge, CH2, CH3** (nucleotide sequence)

tctgatcaggagcccaaatctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtccttc  
ctctccccccaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaaga  
ccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacag  
cacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaag  
ccctccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatccc  
gggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagag  
caatgggcagccggagaacaactacaagaccacgcctcccggtgtggactccgacggctccttctctctacagcaagctcaccgt



ggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc  
ctctccctgtctccgggtaaatactctaga

AA

**HuIgG1 wild type hinge, CH2, CH3 (amino acid sequence)**

5 SDQEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED  
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW  
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY  
TQKSLSLSPGK

10 NT

**Llama IgG1 hinge, CH2, CH3 (nucleotide sequence)**

tgatcaagaaccacatggaggatgcacgtgccncagtgccncaatgccngcncngaactnccaggaggcccttctgtctttg  
tcttcccccgaaaccaaggacgtcctctccattttggaggccgagtcacgtgcgtttagtgagcgtcggaaagaaagaccccg  
aggtcaattcaactggtatattgatggcgttgaggtgcgaacggccaatacgaagccaaaagaggaacagttcaacagcacgtacc  
15 gcgtggtcagcgtcctgcccattccagcaccaggactggctgacggggaaggaattcaagtgaaggtaacaacaaagctctccc  
ggcccccatcgagaggaccatctccaaggccaaagggcagaccgggagccgcaggtgtacacctggccccacaccgggaa  
gaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctaccagctgacatcaacgttgagtggcagaggaacg  
gtcagccggagtcagagggcacctacccaacacgccccacagctggacaacgacgggacctaactctctacagcaagctctc  
ggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggccctgcacaaccactacaccagaaa  
20 tccatcaccagctcttcgggtaaatactctaga

AA

**Llama IgG1 hinge, CH2, CH3 (in figure 23 as Llama IgG1) (amino acid sequence)**

EPHGGCTCPQCPAPELLPGGPSVFVFPPKPKDVLSISGRPEVTCVVVDVGKEDPEVNF  
NWYIDGVEVRTANTKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPA  
25 PIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQP  
ESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHYTQKS  
ITQSSGK

NT

**Llama IgG2 (nucleotide sequence)**

tgatcaagaaccaagacaccaaaccacaaccacaaccacaaccacaaccaatcctacaacagaatccaagtgcccaaatgtc  
cagcccctgagctcctgggaggggccctcagttctcatcttcccccgaaaccaaggacgtcctctccatttctgggaggcccgagg  
5 tcacgtgcgttggttagacgtgggccaggaagaccccgaggcagtttcaactggtacattgatggcgctgaggtgcgaacggcc  
aacacgaggccaaaagaggaacagttcaacagcacgtaccgcgtggtcagcgtcctgcccattccagcaccaggactggctgacg  
gggaaggaattcaagtgaaggtaacaacaagctctccggccccatcgagaagaccatctccaaggccaaaggcgagacc  
cgggagccgcaggtgtacacctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacatgcctggtcaaagg  
cttctaccacctgatatcaacgttgagtgccagaggaatgggcagccggagtcagagggcacytaccgccaccacgccaccccg  
10 ctggacaacgacgggacctacttctctacagcaagctctcgggggaaagaacacgtggcagcaggggagaaaccttcacctgtgt  
ggtgatgcacgaggccctgcacaaccactacaccagaaatccatcaccagtccttcgggtaaataagtaattctaga

AA

**Llama IgG2 (amino acid sequence)**

DQEPKTPKPQPQPQPQNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLISGRPEVT  
15 CVVVDVGQEDPEVSFNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLT  
GKEFKCKVNNKALPAPIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKG  
FYPPDINVEWQRNGQPESEGTATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTC  
VVMHEALHNHYTQKSITQSSGK

NT

20 **Llama IgG3 Fc (nucleotide sequence)**

tgatcaagcgcaccacagcgaagaccccgctccaagtgcccaaatgccaggccctgaactccttgaggggccacgggtcttca  
tcttcccccgaaagccaaggacgtcctctccatcaccggaaaacctgaggtcacgtgcttggtggacgtgggtaaagaagaccc  
tgagatcgagttcaagctggtccgtggatgacacagaggtacacacggctgagacaaagccaaaggaggaacagttcaacagcac  
gtaccgcgtggtcagcgtcctgcccattccagcaccaggactggctgacgggggaaggaattcaagtgaaggtaacaacaagct  
25 ctcccgccccatcgagaggacctctccaaggccaaagggcagaccgggagccgcaggtgtacacctggccccacaccg  
ggaagagctggccaaggacaccgtgagcgtaacctgcctggtcaaaggcttctccagctgacatcaacgttgagtggcagagga  
atgggcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttctctacagcaaac  
tctccgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtggtgatgcacgaggctctacacaatcactccaccag  
aaatccatcaccagtccttcgggtaaataagtaattctagaggggcc

AA

**Llama IgG3 Fc (amino acid sequence)**

DQAHHSEDPSSKCPKCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKT  
LRSSSSWSVDDTEVHTAETKPKKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNK  
5 ALPAPIERTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQR  
NGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHS  
TQKSITQSSGK

NT

**HuIgG1 wild type hinge (nucleotide sequence)**

10 gatcaggagcccaaatcttgacaaaactcacacatgccaccgtgccagca

AA

**HuIgG1 wild type hinge (amino acid sequence)**

DQEPKSCDKTHTCPPCPA

NT

15 **HuIgG1 H2, wild type hinge with leu at second position (nucleotide sequence)**

gatctggagcccaaatcttgacaaaactcacacatgccaccgtgccagca

AA

**HuIgG1 H2, wild type hinge with leu at second position (amino acid sequence)**

DLEPKSCDKTHTCPPCPA

20 **NTHuIgG1 wild type CH2 (nucleotide sequence)**

cctgaactcctggggggaccgtcagttcttcttcccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacat  
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtagcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa  
ggagtacaagtgcaaggtctccaacaagccctcccagccccatcgagaaaaccatctccaaagccaaa

25 AA

**HuIgG1 wild type CH2(amino acid sequence)**

PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA  
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

NT

**HuIgG1 wild type CH3 (nucleotide sequence)**

5 gggcagccccgagaaccacaggtgtacaccctgccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcctg  
gtcaaaggcttctatcccagcgacatcgccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctccc  
gtgctggactccgacggctccttctctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgct  
ccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA

10 **HuIgG1 wild type CH3 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
VLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

**HuIgG1 mutated hinge (C-C-C→S-S-S) (nucleotide sequence)**

15 gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagca

AA

**HuIgG1 mutated hinge (C-C-C→S-S-S) (amino acid sequence)**

DQEPKSSDKTHTSPSPA

NT

20 **HIgG1MTH WTCH2CH3 (mutant hinge with wild type CH2 and CH3 (reads from the hinge+Ig tail) (nucleotide sequence)**

tgatcaccccaaatcttctgacaaaactcacacatctccaccgtcctcagcacctgaactcctgggtggaccgtcagttcttcttccc  
cccaaaacccaaggacacctcatgatctcccgaccctgaggtcagatgcgtggtggagcgtgagccacgaagaccctgag  
gtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtac  
25 cgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccc  
agcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggtatga  
gctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagagcaatggg

cagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttcttctctacagcaagctcaccgtggacaa  
gagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccc  
tgtctccgggtaaatgataatctaga

AA

5 **Mutant hinge, but wild type CH2 and CH3 (amino acid sequence)**

DHPKSSDKTHTSPSSAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE  
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN  
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES  
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQ

10 KSLSLSPGK

NT

**LLG1-5'bgl 35 mer Llama IgG1 5'**

5'-gtt gtt gat caa gaa cca cat gga gga tgc acg tg-3'

NT

15 **LLG2-5'bgl 32 mer, Llama IgG2-5'**

5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

NT

**LLG3-5'bgl 33 mer, Llama IgG3-5'**

5'-gtt gtt gat caa gcg cac cac agc gaa gac ccc-3'

20 NT

**LLseqsense 19mer, llama sequencing primer**

5'-ctg aga tcg agt tca gct g-3'

NT

**LLseqAS 19 mer**

25 5'-cct cct ttg gct ttg tct c-3'

NT

### **2H7 scFv llama IgG1 (nucleotide sequence)**

aagcttgccgccatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
5 gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
10 atctgaagactctcggtctatttctgtgcaagagtggtgtactatagtaacttacttggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcaagaaccacatggaggatgcacgtgccncagtgccncaatgccngcncngaactnccagga  
ggccttctgtcttcttcccccgaaaccaaggacgtccttccattttggaggccgagtcacgtgcgttgtagtgagcgtcgga  
aagaaagaccccgaggtcaatttcaactggtatattgatggcggtgaggtgcgaacggccaatacgaagccaaaagaggaacagtt  
caacagcacgtaccgctggtcagcgtctgccccatccagcaccaggactggctgacggggaaggaaattcaagtgaaggtcaac  
15 aacaaagctctcccgcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacacctggc  
cccacaccgggaagaactggccaaggacaccgtgagcgtaacatgcctggtcaaaggcttctaccagctgacatcaacgttgagt  
ggcagaggaacgggtcagccggagtcagagggcacctacgccaacacgccgccacagctggacaacgacgggacctacttctct  
acagcaagctctcgggtgggaaagaacacgtggcagcggggagaaaccttaacctgtgtggtgatgcatgaggcctgcacaacca  
ctacaccagaaatccatcaccagctcttcgggtaaatagtaattctaga

20 AA

### **2H7 scFv llama IgG1 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
25 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPHGGCTCPQCP  
APELPGGPSVFVFPKPKDVLSIFGGRVTCVVVDVGKKDPEVNFNWDYIDGVEVRTA  
NTKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTR

EPQVYTLAPHREELAKDTVSVTCLVKGFYPADINVEWQRNGQPESEGTYANTPPQL  
DNDGTYFLYSKLSVGKNTWQRGETLTCVVMHEALHNHYTQKSITQSSGK

NT

**2H7 scFv llama IgG2 (nucleotide sequence)**

5 aagcttgccgcatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttcagggccagctcaagtgttaattacatgcactgg  
taccagcagaagccaggatcctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctgggcgggtggatctggaggaggtgggagc  
10 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgctcgtcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtactatagtaactcttacttggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaagaacccaagacaccaaaccacaaccacaaccacaacccaatcctacaacagaatcca  
15 agtgtcccaaatgtccagcccctgagctcctgggagggccctcagttctcatcttcccccgaaacccaaggacgtcctctccatttct  
gggaggccccgaggtcacgtgcgttggttagacgtgggccaggaagaccccgaggtcagtttcaactggtacattgatggcgctg  
aggtgcgaacggccaacacgaggccaaaagaggaacagttaacagcacgtaccgcgtggtcagcgtcctgccccatccagcacc  
aggactggctgacggggaaggaattcaagtcaaggtcaacaacaaagctctccggcccccatcagagaagaccatctccaaggc  
caaagggcagacccgggagccgaggtgtacacctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacat  
20 gcctggtcaaaggcttctacccacctgatatcaacgttgagtgagcagaggaatgggcagccggagtcagagggcacgtacgccac  
cacgccacccagctggacaacgacgggacctacttctctacagcaagctctcggtgggaaagaacacgtggcagcagggaga  
aaccttcacctgtgtggtgatgcagaggccctgcacaaccactacaccagaaatccatcaccagctcttcgggtaaatagtaatct  
aga

**AA2H7 scFv llama IgG2 (amino acid sequence)**

25 MDFVQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKTPKPQPQPQP

QPNPTTESKCPKCPAPELLGGPSVFIFPPKPKDVLSISGRPEVTCVVVDVGQEDPEVS  
FNWYIDGAEVRTANTRPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALP  
APIEKTISKAKGQTREPQVYTLAPHREELAKDTVSVTCLVKGFYPPDINVEWQRNG  
QPESEGTYATTPPQLDNDGTYFLYSKLSVGKNTWQQGETFTCVVMHEALHNHYTQ  
5 KSITQSSGK

**NT2H7 scFv llama IgG3 (nucleotide sequence)**

aagcttgccgccatggattttcaagtcagattttcagcttcctgctaatacagtcctcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgaagttacatgcactgg  
taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga  
10 gtgggtctgggaccttactctctcacaatcagcagatggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtgctcggcggtggtgatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
15 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaagcgaaccacagcgaagaccccagctccaagtgcccaaatgccaggccctgaactccttgag  
ggcccacggtcttcatcttcccccgaaagccaaggacgtcctctccatcacccgaaaacctgaggtcacgtgcttgtggtggacgt  
gggtaagaagaccctgagatcgagttcaagctggctcgtggatgacacagaggtacacacggctgagacaaagccaaaggagg  
aacagttcaacagcacgtaccgcgtggtcagcgtcctgcccattcagcaccaggactggctgacggggaaggaattcaagtcaa  
20 ggtcaacaacaaagctctcccagcccccatcgagaggaccatctccaaggccaaagggcagacccgggagccgcaggtgtacac  
cctggccccacaccgggaagagctggccaaggacaccgtgagcgtaacctgcctggtcaaaggcttctcccagctgacatcaac  
gttgagtggcagaggaatgggcagccggagtcagaggcacctacgccaacacgccgccacagctggacaacgacgggaccta  
cttctctacagcaaactctcgtgggaaagaacacgtggcagcagggagaagtcttcacctgtgtggtgatgcacgagggtctaca  
caatcactccaccagaaatccatcacccagctctcgggtaaatagtaattctagagggcc

25 AA

**2H7 scFv llama IgG3 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG



YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQAHSHSEDPSSKCP  
 KCPGPELLGGPTVFIFPPKAKDVLSITRKPEVTCLWWTWVKKTLRSSSSWSVDDTEV  
 HTAETKPKEEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNKALPAPIERTISKAKG  
 5 QTREPQVYTLAPHREELAKDTVSVTCLVKGFFPADINVEWQRNGQPESEGTYANTP  
 PQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

NT

**2H7+Completely WT IgG tail (nucleotide sequence)**

**2H7 scFv WTH WTCH2CH3 (nucleotide sequence)**

10 aagcttgccgcatggatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
 ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga  
 gtgggtctgggaccttactcttcacaatcagcagagtggaggctgaagatgtgccacttattactgccagcagtgaggtttaac  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
 15 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact  
 tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaacttctactggtacttcgatgtctggggcacagggaccac  
 ggtcaccgtctcttctgatcaggagcccaatctgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggg  
 20 accgtcagttctcttccccccaaaaccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtggagctg  
 agccacgaagaccctgaggtcaagtcaactgttacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggag  
 cagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggt  
 ctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct  
 gccccatcccggtgatgactgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtgg  
 25 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagc  
 aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
 gcagaagagcctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv WTH WTCH2CH3 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHYQ  
 QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
 PPTFGAGTKLELKDGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 5 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTCPP  
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP  
 VLDSGDSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

10 **NTCD80 transmembrane domain and cytoplasmic tail (+restriction sites) (nucleotide sequence)**

gcggatccttgaacctgctcccatcctgggccattaccttaatctcagtaaagggaattttgtgatatgctgcctgacctactgctttgc  
 cccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgat

AA

15 **CD80 transmembrane domain and cytoplasmic tail (amino acid sequence)**  
 ADPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**40.2.220 VL (anti-human CD40 scFv #1--VL) (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactca  
 20 gtctccagccaccctgtctgtgactccaggagatagagtctctcttctcctgcagggccagccagagtattagcgactacttacactgg  
 atcaacaaaaatcacatgagtctccaaggcttctcatcaaatatgcttccattccatctctgggatcccctccaggttcagtggcagtg  
 gatcagggtcagatttcactctcagtatcaacagtggtgaacctgaagatgttggaatttattactgtcaacatgggtcacagctttccgtg  
 gacgttcggtggaggcaccaagctggaaatcaaacgg

AA

25 **40.2.220 VL (anti-human CD40 scFv #1--VL) (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW  
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFP  
WTFGGGTKLEIKR

NT

5 **40.2.220 VH (for anti-human CD40 scFv #1--VH) (nucleotide sequence)**

cagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccttca  
caactactggaatgcagtggtgcaagagatgccaggaaagggttgaagtggattggctggataaacaccccactctggagtgcc  
aaaatatgtagaagacttcaaggacggttgccttctcttggaaacctctgccaactgcataattacagataagcaacctcaaagat  
gaggacacggctacgtatttctgtgtgagatccgggaatgtaactatgacctggcctactttgcttactggggccaagggaactgg  
10 tctactgtctgatca

AA

**40.2.220 VH (for anti-human CD40 scFv #1--VH) (amino acid sequence)**

QIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQEMPGKGLKWIGWINTPL  
WSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYFCVRSNGNGNYDLAYFAY  
15 WGQGTLLTVS

NT

**40.2.220 scFv (anti-human CD40 scFv #1) (nucleotide sequence)**

aagcttatggatttcaagtgcagatttctcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgttctgactca  
gtctccagccaccctgtctgtgactccaggagatagagctctcttctcctgcagggccagccagagtattagcgactacttacactggt  
20 atcaacaaaaatcacatgagttccaaggcttctcatcaaatatgcttccattccatctctgggatcccctccaggttcagtggcagtg  
gatcagggtcagattcactctcagtatcaacagtggtgaacctgaagatgttggaatttactgtcaacatgggtcacagcttccgtg  
gacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggtggcggcggtatc  
agatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccttcac  
aactactggaatgcagtggtgcaagagatgccaggaaagggttgaagtggattggctggataaacaccccactctggagtcca  
25 aaatatgtagaagacttcaaggacggttgccttctcttggaaacctctgccaactgcataattacagataagcaacctcaaagatg  
aggacacggctacgtatttctgtgtgagatccgggaatgtaactatgacctggcctactttgcttactggggccaagggaactgg  
cactgtctgatca

AA

**40.2.220 scFv (anti-human CD40 scFv #1) (amino acid sequence)**

MDFQVQIFSFLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSIDYLHW  
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIIYQCQHGHSFP  
5 WTFGGGTKLEIKRGGGGSGGGGSGGGGSQIQLVQSGPELKKPGETVRISCKASGYA  
FTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQIS  
NLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVS

NT

**2e12 VL (with L6 VK leader peptide) (nucleotide sequence)**

10 atggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgctcacccaatctcc  
agcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaggtgtgaatattatgtcacaagttaatgcag  
tggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggggtccctgccaggttagtg  
gcagtggtgctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagtaggaa  
ggttccttgacgttcggtggaggcaccaagctggaaatcaaacgg

15 AA

**2e12 VL (with L6 VK leader peptide) (amino acid sequence)**

MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL  
MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ  
QSRKVPWTFGGGTKLEIKR

20 NT

**2e12 VH (no leader peptide) (nucleotide sequence)**

cagggtgcagctgaaggagtcaggacctggcctggcggcgcctcacagagcctgtccatcacatgcaccgtctcagggttctcatta  
accggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggtgatggaagcacag  
actataattcagctctcaaataccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaacagctctgcaaact  
25 gatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggggtcaaggaacctcagtc  
accgtctcctca(gatctg)

AA

**2e12 VH (amino acid sequence)**

QVQLKESGPGLVAPSQSL SITCTVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGS  
TDYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTARYYCARDGYSNFHYVMDY  
WGQGTSVTVSS

5 NT

**2e12scFv(+Restriction sites) (nucleotide sequence)**

aagcttatggatttcaagtgcagatttcagcttctgctaatacgtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaagtggtgaatattatgtcacaaagtta  
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtcctgccagg  
10 tttagtggcagtggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtattctgtcagcaaagt  
aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggtcgggagggtgggtcgggt  
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatgggggtg  
atggaagcacagactataattcagctctcaaactcagactgagcatcaccaaggacaactccaagagccaagtttctaaaaatgaa  
15 cagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctcctct(gatcag)

AA

**2e12scFv (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL  
20 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ  
QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSL SITC  
TVSGFSLTGYGVNWVRQPPGKGLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ  
VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGTSVTVSS

NT

25 **10A8 is anti-CD152 (CTLA-4)**

**10A8 VL (with L6 VK leader peptide) (nucleotide sequence)**

atggatttcaagtgcagatttcagcttctgctaatacgtgcttcagtcataatgtccagaggagtcgacatccagatgacacagctctc  
catcctcactgtctgcatctctgggaggcaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttggtacca

acacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaaagtggg  
tctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttcattgac  
gttcggctcggggacaaagttggaataaaacgg

AA

5 **10A8 VL (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIQMTQSPSSLSASLGKVTITCKASQDIKKYIGW  
YQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSRDYSLSIRNLEPEDIATYYCQQYDN  
LPLTFGSGTKLEIKR

NT

10 **10A8 VH (no leader peptide) (nucleotide sequence)**

gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtcctctgtctctcacctgctctgtcactggctactccatcacc  
agtggtttctactggaactggatccgacagttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac  
tacaacctctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttctgaagttgagttctgtgactactgagga  
cacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactgggggtcaaggaacctcagtcaccgtctcctct  
gatca

AA

**10A8 VH (amino acid sequence)**

DVQLQESGPGLVKPSQSLTCSVTGYSITSGFYWNWIRQFPGNKLEWMGHISHDG  
RNNYNPSLINRISITRDTSKNQFFLKLSSVTTEDTATYFCARHYGSSGAMDYWGQGT  
SVTVSS

NT

**10A8 SCFV (nucleotide sequence)**

aagcttatggattttcaagtcagattttcagttcctgctaatacagtcgttcagtcataatgtccagaggagtcgacatccagatgacac  
agttccatcctcactgtctgcatctctgggaggcaaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttg  
taccaacacaagcctggaaaagggtcccaggctgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaa  
gtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttcc  
attgacgttcggctcggggacaaagttggaataaaacggggtggcgggtggctcgggcggtgggtgggtcgggtggcggcggtatct

gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtctctgtctctcacctgctctgtcactggctactccatcacc  
agtggtttctactggaactggatccgacagtttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac  
tacaacctatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgagga  
cacagctacatatttctgtgcaagacactacggtagtagcggagctatggactactgggggtcaaggaacctcagtcaccgtctctct  
5 gatca

AA

**10A8 SCFV (amino acid sequence)**

MDFQVQIFSFLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIGW  
YQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGSGRDYSLIRNLEPEDIATYYCQQYDN  
10 LPLTFGSGTKLEIKRGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSVTGY  
SITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFLKLSS  
VTTEDTATYFCARHYGSSGAMDYWGQGTSVTVSSD

NT

**40.2.220-hm1IgG1-hCD80 (nucleotide sequence)**

15 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgttctgactca  
gtctccagccaccctgtctgtgactccaggagatagagtctcttctcctgcagggccagccagagtattagcgactacttacactgg  
atcaacaaaaatcacatgagttctccaaggcttctcatcaaatatgcttccattccatctctgggatcccctccaggttcagtggcagtg  
gatcagggtcagatttactctcagtatcaacagtggtgaacctgaagatgttggaatttattactgtcaacatggtcacagctttccgtg  
gacgttcgggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggcggaggtgggtcgggtggcggcggatctc  
20 agatccagttgggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaaggcttctgggtatgccttac  
aactactggaatgcagtggtgcaagagatgccaggaaagggttgaagtggattggctggataaacaccccactctggagtcca  
aaatatgtagaagacttcaaggacggtttgccttctctttggaacctctgccaacactgcataattacagataagcaacctcaaagatg  
aggacacggctacgtatttctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaagggacactgg  
cactgtctctgatctggagcccaaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtca  
25 gtcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggtgacgtgagccac  
gaagacctgaggtcaagttcaactgggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtac  
aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtctccaa  
caaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc  
atcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtggg

agagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctc  
accgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcaga  
agagcctctccctgtctccgggtaaagcggatccttgaacctgctcccatcctgggccattacctaatctcagtaaatggaattttgt  
gatatgctgctgacctactgctttgcccccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctg  
5 tataaatcgat

AA

**40.2.220-hmIgG1-hCD80 (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHW  
YQQKSHESPRLLIKYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFP  
10 WFTGGGKLEIKRGGGGSGGGGSGGGGSGGGGSIQLVQSGPELKKPGETVRISCKASGYA  
FTTTGMQWVQEMPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQIS  
NLKDEDTATYFCVRSGNGNYDLAYFAYWGQGTLVTVSDLEPKSSDKTHTSPSPAP  
ELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK  
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
15 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD  
GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPSNLLPSW  
AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**2e12scFv- hmIgG1-CD80 fusion protein (nucleotide sequence)**

20 aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcaccaacgtagaatctggggtcctgccagg  
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
aggaaggttcttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt  
25 ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaa  
cagctctgcaaactgatgacacagccagataactactgtgccagagatgggtatagtaactttcattactatgttatggactactggggtcaa  
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc



tggggggatcgtcagttcttcttcccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtgggtggt  
 ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
 ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaag  
 tgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt  
 5 acaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatc  
 gccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
 cactacacgcagaagagcctctccctgtctccgggtaaagcggtaccttgaacctgctcccatcctgggccattacctaatactcagt  
 aaatggaattttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaaggga  
 10 aagtgtacgccctgtataaatcgat

AA

**2e12scFv- hmtIgG1-CD80 fusion protein (amino acid sequence)**

MDFVQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL  
 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ  
 15 QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLITC  
 TVSGFSLTGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSITKDNSKSQ  
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWQGTSVTVSSDLEPKSSDKT  
 HTSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  
 20 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKA  
 DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**10A8 scFv-hmtIgG1-CD80 (nucleotide sequence)**

25 aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac  
 agtctccatcctcactgtctgcatctctgggaggcaaaagtcacatcacttgcaaggcaagccaagacattaagaagtatataggttgg  
 taccaacacaagcctggaaaaggtcccaggctgctcatatattacacatctacattacagccaggcacccatcaagggtcagtgga  
 gtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttc  
 attgacgttcggctcggggacaaagttggaaataaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc

gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtcctgtctctcacctgctctgtcactggctactccatcacc  
 agtggtttctactggaactggatccgacagttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac  
 tacaacctatctctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgagga  
 cacagctacataatttctgtgcaagacactacggtagtagcggagctatggactactggggtaaggaacctcagtcaccgtctcctct  
 5 gatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagttctcctct  
 cccccaaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgggacgtgagccacgaagaccct  
 gaggtcaagttcaactggtagcgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg  
 taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccct  
 ccagcccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatccggga  
 10 tgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagcaatg  
 ggcagccgggagaacaaactacaagaccacgcctcccgtgctggactccgacggctccttctcctctacagcaagctcaccgtggac  
 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
 cctgtctccgggtaaagcggatccttcgaacctgtcccatcctggggcattaccttaatctcagtaaattggaattttgtgatatgctgc  
 ctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgcctgtataaatcga  
 15 t

AA

**10A8 scFv-hmIgG1-CD80 (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIQMTQSPSSLSASLGGKVTITCKASQDIKKYIGW  
 YQHKPGKGPRLLIYYTSTLQPGIPSRFSGSGGRDYSLSIRNLEPEDIATYYCQQYDN  
 20 LPLTFGSGTKLEIKRGGGSGGGGSGGGGSDVQLQESGPGLVKPSQSLSLTCSVTGY  
 SITSGFYWNWIRQFPGNKLEWMGHISHDGRNNYNPSLINRISITRDTSKNQFFLKLSS  
 VTTEDTATYFCARHYGSSGAMDYWGQGTSTVTVSSDLEPKSSDKTHTSPSPAPELL  
 GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP  
 REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ  
 25 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF  
 FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKADPSNLLPSWAIT  
 LISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**500A2-hmtIgG1-CD80 (nucleotide sequence)**

atgttgatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtgacatagtctgactcagactccagccactct  
gtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaattctacactgggtatcaccaaaaaccaa  
aggaggctccaagggctctcatcaagtatgcttcgcagtccattcctgggatccctccagattcagtggcagtggttcggaacaga  
5 tttactctcagcatcaataacctggagcctgatgatatcggaatttactgtcaacaaagtagaagctggcctgtcacgttcggtcct  
ggcaccaagctggagataaaacggggtggcgggtggtcgggcggaggtgggtcgggtggcggcggatctcaggtcaagctgca  
gcagtcgggttctgaactagggaaacctggggcctcagtgaactgtcctgcaagacttcaggctacatattcacagatcactatatt  
cttgggtgaaacagaagcctggagaaagcctgcagtgataggaaatgtttatggtggaaatggtgtacaagctacaatcaaaaatt  
ccaggggcaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgaggattctgccat  
10 ctattactgtgcaagaaggccggtagcgacgggcatgctatggactactggggtcaggggatccaagttaccgtctcctctgatctg  
gagcccaaatcttctgacaaaactcacacatccccaccgtcccagcacctgaactcctggggggatcgtcagttctctcttcccc  
caaaaccaagggacacctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggt  
caagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaaagccgcgggaggagcagtacaacagcacgtaccg  
tgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggttccaacaaagccctccag  
15 ccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtatgagc  
tgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggca  
gccggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctcctctacagcaagctcaccgtggacaaga  
gcaggtggcagcaggggaacgtcttctcatgctccgtgatgcaggtctgcacaaccactacacgcagaagagcctctccctgt  
ctccgggtaaagcggatccttgaacctgtcccatcctgggccattacctaatactcagtaaatggaattttgtgatgtcctgac  
20 ctactgctttgccccagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgcctgtataaatcgat

AA

**500A2-hmtIgG1-CD80 (amino acid sequence)**

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYHQ  
KPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPVTF  
25 GPGTKLEIKRGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIFTD  
HYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMELSSLT  
SEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSDLEPKSSDKTHTSPSPAPELLGG  
SSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY

TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL  
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSWAITLI  
SVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

5 **2H7 scFv MTH(SSS)WTCH2CH3 (nucleotide sequence)**

aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgaagttacatgactgg  
taccagcagaagccaggatcctcccccacccctggatttatgcccacccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggacctcttactcttcacatcagcagagtgaggagctgaagatgctgccacttattactgccagcagtgagggtttaac  
10 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtatact  
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcacagggaccac  
15 gggtaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga  
ccgtcagctcttcttctccccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgggtggacgtga  
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
20 ccccatcccggtgatgactgaccaagaaccaggtcagcctgacctgctggtaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagacaactacaagaccacgcctcccgctgctggactccgacggctccttcttctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgttctctatgctccgtgatgcatgaggctctgcacaaccactacag  
cagaagagcctctccctgtctccgggtaaatgatctaga

AA

25 **2H7 scFv MTH(SSS)WTCH2CH3 (amino acid sequence)**

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM

QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP  
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
5 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

NT

**HuIgGMHncs1 (oligo for CSS) (nucleotide sequence)**

ggt ggt gat cag gag ccc aaa tct tgt gac aaa act cac

NT

10 **HuIgGMHncs2 (oligo for SCS=ncs2) (nucleotide sequence)**

ggt ggt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg

NT

**HuIgGMHncs3 (oligo for SSC=ncs3) (nucleotide sequence)**

ggt ggt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca gca cct g

15 NT

**hIgGWT3xba (3' oligo for above mutation introduction) (nucleotide sequence)**

ggt ggt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

NT

**Vhser11: (oligo for Leu to Ser at VH11) (nucleotide sequence)**

20 **gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tcg gtg agg cc**

NT

**huIgG1-3' (3' oligo to amplify IgG1 C regions, 3' end of CH3) (nucleotide sequence)**

gtc tct aga cta tca ttt acc cgg aga cag

NT

25 **huIgA/Gchim5 (oligo for pcr#1) (nucleotide sequence)**

cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg

NT

**huIgAhg-5' (oligo for pcr#2) (nucleotide sequence)**

ggt ggt gat cag cca gtt ccc tca act cca cct acc cca tct ccc caa ct

NT

5 **huIgA3' (nucleotide sequence)**

ggt ggt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac

NT

**2H7 scFv IgAH IGG WT CH2CH3 (2H7 scFv with IgA hinge and WT CH2 and CH3)**  
**(nucleotide sequence)**

10 aagcttgccgcatggattttcaagtgcagattttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggttttaac  
cccccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
15 tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtatact  
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactgggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgcgcacctga  
20 actcctggggggaccgtcagttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgt  
gggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaagacaaag  
ccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagt  
acaagtgaaggtctccaacaaagccctccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccac  
aggtgtacacctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccagc  
25 gacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtgctggactccgacggctcc  
ttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgaggctctgc  
acaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv IgAH IGG WT CH2CH3 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
5 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSDQVPSTPPTPSPSTPP  
TPSPSCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG  
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS  
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
10 TPPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

**2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgAhinge and IgA CH2 and CH3) (nucleotide sequence)**

aagcttgccgccatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
15 ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgaagtacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggacctcttactcttcacaatcagcagagtgaggagctgaagatgctgccacttattactgccagcagtgagggtttaac  
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaatgctcgaaggcttctggctacacat  
20 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggatgact  
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctctctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgctgccac  
ccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag  
25 agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg  
ctacagcgtgtccagtgctctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctaccccgagtc  
caagaccccgtaccgccacctctcaaatccggaaacacattccggcccaggtccacctgctgccgccgctcgaggag  
ctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaggtgtgctggttcgctgggtgcaggggctc  
acaggagctgccccgcgagaagtacgtgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag

catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttc  
acacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacggcgaggtggacggcacctgctac  
tgataatctaga

AA

5 **2H7 scFv IgAH IgACH2CH3** (2H7 scFv IgA hinge and IgA CH2 and CH3) (amino acid sequence)

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
10 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQVPSTPPTPSPSTP  
PTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQG  
PPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEV  
HLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ  
15 GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSV  
VMAEVDGTCY

NT

**IgA hinge-CH2-CH3** (Human IgA tail, full length) (nucleotide sequence)

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccaccccgactgtcact  
20 gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg  
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca  
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcctaccccgagtccaagaccccgcta  
accgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctggccctgaacg  
agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc  
25 cgcgagaagtacgtgacttgggcatcccgagcagggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg  
cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttcacacagaagacca  
tcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgttgcacggcgaggtggacggcacctgctactgataatctaga

AA



**IgA hinge-CH2-CH3 (Human IgA tail, full length) (amino acid sequence)**

DQVPVSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT  
FTWTPSSGKSAVQGGPDRDLGCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL  
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP  
5 REKYLTWASRQEPSQGTTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKT  
IDRLAGKPTHVNVSVVMAEVDGTCY

NT

**Human J Chain (nucleotide sequence)**

10 agatctcaagaagatgaaaggattgttcttggtgacaacaaatgtaagtgtgcccgattacttcaggatcatccgttctccgaagatc  
ctaagaggacattgtggagagaaacatccgaattattgtcctctgaacaacaggaggagaatatctctgatccacctcaccattgaga  
accagatttgtgtaccatttgtctgacctcagctgtaaaaaatgtatcctacagaagtggagctggataatcagatagttactgctacc  
agagcaatatctgtgatgaagacagtgctacagagacctgtacacttatgacagaaacaagtgtacacagctgtgggtccactcgt  
atatgggtggtgagacaaaatggtggaaacagccttaacccagatgcctgctatcctgactaatctaga

15 AA

**Human J Chain (amino acid sequence)**

RSQEDERIVLVDNKCKCARITSRIIRSSDPNEDIVERNIRIIVPLNNRENISDPTSPLRT  
RFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVV  
PLVYGGETKMOVETALTPDACYP

20 NT

**HUJCH5nl (J chain 5' primer) (nucleotide sequence)**

ggt ggt aga tct caa gaa gat gaa agg att ggt ctt

NT

**HUJCH3 (J chain 3' primer-antisense) (nucleotide sequence)**

25 ggt ggt tct aga tta gtc agg ata gca ggc atc tgg

AA

**4 carboxy terminal amino acids deleted from IgA CH3 (amino acid sequence)**

GTCY

NT

**IgAH IgAT4** (human IgA tail, truncated (3T1)-(missing last 4 amino acids from carboxy terminus) (nucleotide sequence)

5   tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact  
gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg  
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca  
gtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcactgctgcctaccccgagtccaagaccccgcta  
accgccaccctctcaaaatccggaacacattccggcccgaggtccacctgctgccgccgccgtcgaggagctggccctgaacg  
10 agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc  
cgcgagaagtacgtgactgggcatcccgagcagggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg  
cagccgaggactggaagaagggggacaccttctctgcatggtgggccacgaggccctgccgctggccttcacacagaagacca  
tcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcacgtggcgagggtggactgataatctaga

AA

15   **IgAH IgAT4** (amino acid sequence)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT  
FTWTPSSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL  
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP  
REKYLTWASRQEPSQGTTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKT  
20 IDRLAGKPTHVNVSVVMAEVD

NT

**HUIGA3T1** (oligo 3' to delete 4 amino acids at carboxy end of IgA CH3)

ggt gtt tct aga tta tca gtc cac ctc cgc cat gac aac aga cac

NT

25   **HUIGA3T2** (oligo to delete 14 aa at end of IgA –T4)

ggt gtt tct aga tta tca ttt acc cgc caa gcg gtc gat ggt ctt

NT

**2H7 scFv IgAH IgAT4 (2H7 scFv IgA 3T1 construct; truncates the CH3 domain at the 3'end) (nucleotide sequence)**

aagcttgccgcatggattttcaagtcagattttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagtctccagcaatcctgtctgcatctccaggggagaaggtcaccaatgacttgaggccagctcaagtgaagttacatgcactgg  
5 taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggagtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
10 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttacttggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccac  
ccccgactgtcactgcaccgaccggccctcaggacactgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag  
agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg  
15 ctacagcgtgtccagtgctctgccgggctgtgccgagccatggaacctgggaagaccttcaactgactgctgcctaccccgagtc  
caagaccccgctaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggag  
ctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctgggtcgtggctgcaggggtc  
acaggagctgccccgcgagaagtacgtgacttgggcacccggcaggagcccagggcaccaccaccttcgctgtgaccag  
catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttc  
20 acacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcacgtggcgagggtggactgataatctaga

AA

**2H7 scFv IgAH-T4 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
25 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSSDQVPSTPPTPSPSTP  
PTPSPSCCHPRLSLHRPALEDLLGSEAILTCTLTGLRDASGVTFTWTPSSGKSAVQG  
PPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEV

HLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQ  
GTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSV  
VMAEVD

AA

- 5 **14 amino acids deleted from IgAH-T4** (so that total of 18 amino acids deleted from wild type IgA CH3) (amino acid sequence)

PTHVNVSVVMAEVD

NT

**IgAH IgA-T18** (human IgA Tail truncated, 3T2) (nucleotide sequence)

- 10 tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact  
gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg  
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca  
gtgtctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcctaccccgagtccaagacccccgcta  
accgccaccctctcaaaatccggaacacattccggcccgaggccacctgctgccgcccgccgctggaggagctggccctgaacg  
15 agctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccc  
cgcgagaagtacgtgacttgggcatcccgagcaggagcccagccagggcaccaccaccttcgctgtgaccagcactgctgcgctgg  
cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagacca  
tcgaccgcttggcgggtaaa

AA

- 20 **IgAH IgA-T18** (amino acid sequence)

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT  
FTWTPSSGKSAVQGPPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL  
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP  
REKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKT

- 25 IDRLAGK

NT

**2H7 scFv IgAH IgAT18** (human IgA Tail truncated, 3T2) (nucleotide sequence)

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaattacatgcactgg  
 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
 5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac  
 10 ggtcaccgtctcttctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccac  
 ccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag  
 agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg  
 ctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgcctaccccgagtc  
 caagaccccgctaaccgccaccctctcaaatccggaaacacattccggcccaggtccacctgctgccgccgccgtcgaggagg  
 15 ctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtc  
 acaggagctgccccgcgagaagtacctgactgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag  
 catactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggccacgaggccctgccgctggccttc  
 acacagaagaccatcgaccgcttggcgggtaaa

AA

20 **2H7 scFv IgAH IgAT18 (amino acid sequence)**

MDFVQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 25 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQVPSTPPTPSPSTP  
 PTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSGKSAVQG  
 PPDRDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEV  
 HLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQ  
 GTTTFVAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGK

NT

**CTLA-4 IgG WTH WTCH2CH3 (human-oncoMLP-CTLA4EC-hIgGWT) (nucleotide sequence)**

gcaacctacatgatggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactat  
5 ccaaggactgagggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggca  
acggaacccagatttatgtaattgatccagaaccgtgcccagattctgatcaacccaaatcttgacaaaactcacacatgccaccg  
tgcccagcacctgaactcctggggggaccgtcagttctctcttccccccaaaacccaaggacaccctcatgatctccggaccct  
gaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcata  
atgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggct  
10 gaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggca  
gccccgagaaccacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaa  
ggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccggtctg  
gactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtg  
atgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

15 AA

**CTLA-4 IgG WTH WTCH2CH3 (amino acid sequence)**

MGVLLTQRTL LSLVLALLFPSMASMAMHVAQPAVV LASSRGIASFVCEYASPGKAT  
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM  
DTGLYICKVELMYPPPYL LGIGNGTQIYVIDPEPCPDSDQPKSCDKTHTCPPCPAPEL  
20 LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK  
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP  
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS  
FFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

NT

25 **Human OncoM leader Peptide+CTLA4 EC (BclII) (nucleotide sequence)**

atgggggtactgctcacacagaggacgctgctcagctggtccttgccactcctgtttccaagcatggcgagcatggcaatgcacgtg  
gccagcctgctgtggtactggccagcagccgaggcatgccagctttgtgtgtagtatgcattccaggcaaagccactgaggtc  
cgggtgacagtgcttcggcaggctgacagccaggtgactgaagctctgtcggcaacctacatgatggggaatgagttgaccttcta  
gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatggacacgggactctac

atctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt  
gccagattctgatcaa

AA

**Human OncoM leader Peptide+CTLA4 EC (amino acid sequence)**

5 MGVLLTQRTLTLVLALLFPSMASMAMHVAQPAVVLASSRGIA SFVCEYASPGKAT  
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM  
DTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQ

NT

**Human OncoM leader (nucleotide sequence)**

10 atgggggtactgctcacacagaggacgctgctcagctctggctcctgcactcctgtttccaagcatggcgagcatg

AA

**Human OncoM leader (amino acid sequence)**

MGVLLTQRTLTLVLALLFPSM

NT

15 **Human CTLA4 EC (no LP) (nucleotide sequence)**

gcaatgcacgtggcccagcctgctgtgtgactggccagcagccgaggcatgccagcttgtgtgtgagtatgcatctccaggcaaa  
gccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtgcggcaacctacatgacggggaatg  
agttgaccttcctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaaggactgagggccatgga  
cacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctgggcataggcaacggaaccagatttatgtaatt  
20 gatccagaaccgtgccagattct

AA

**Human CTLA4 EC (no LP) (amino acid sequence)**

AMHVAQPAVVLASSRGIA SFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMT  
GNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYLIGINGTQ  
25 IYVIDPEPCPDS

NT

**Human CTLA4 IgG MTH (SSS) MTCH2CH3 (nucleotide sequence)**

atgggggtactgctcacacagaggacgctgctcagtctggctcttgcaactctgttccaagcatggcgagcatggcaatgcacgtg  
gcccagcctgctgtggtactggccagcagccgagggcatgccagctttgtgtgtgagtatgcatctccaggcaaagccactgaggtc  
cgggtgacagtgttcggcaggtgacagccaggtgactgaagtctgtcggcaacctacatgatggggaatgagttgaccttcta  
5 gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac  
atctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt  
gcccagattctgatcaacccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcctggggggatcgtcagt  
cttctcttcccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggacgtgagccacga  
agacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagcagtagaa  
10 cagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaaca  
aagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctgccccat  
ccgggatgagctgaccaagaaccaggtcagcctgacctgctgctcaaaggcttctatcccagcgacatcgccgtggagtggga  
gagcaatgggcagccggagaacaactacaagaccacgcctccgtgctggactccgacggctcttctctctacagcaagctca  
ccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaa  
15 gagcctctccctgtctccgggtaaatga

AA

**Human CTLA4 IgG MTH (SSS) MTCH2CH3 (amino acid sequence)**

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVCEYASPGKAT  
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM  
20 DTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDSQPKSSDKTHTSPSPAPELL  
GGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP  
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ  
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF  
FLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

25 NT

**CTLA-4 IgAH IgACH2CH3 (human-oncoMLP-CTLA4EC-IgA) (nucleotide sequence)**

atgggggtactgctcacacagaggacgctgctcagtctggctcttgcaactctgttccaagcatggcgagcatggcaatgcacgtg  
gcccagcctgctgtggtactggccagcagccgagggcatgccagctttgtgtgtgagtatgcatctccaggcaaagccactgaggtc  
cgggtgacagtgttcggcaggtgacagccaggtgactgaagtctgtcggcaacctacatgatggggaatgagttgaccttcta



gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac  
 atctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt  
 gccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccc  
 gactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagat  
 5 gcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacctctgtggctgtac  
 agcgtgtccagtgtctgccgggctgtgccgagccatggaaccatgggaagaccttcacttgactgctgcttaccgccagtcgaag  
 accccgctaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctgg  
 ccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacag  
 gagctccccgcgagaagtacctgactgggcatcccgccaggagcccagggcaccaccaccttcgctgtgaccagcata  
 10 ctgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgtggccttcaca  
 cagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcacggcgagggtggacggcacctgctactga  
 taatctaga

AA

**CTLA-4 IgAH IgACH2CH3 (amino acid sequence)**

15 MGVLLTQRTLTLVLALLFPSMASMAMHVAQPAVVLASSRGIA SFVCEYASPGKAT  
 EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM  
 DTGLYICKVELMYPPPYLIGINGTQIYVIDPEPCPDS DQVPSTPPTPSPSTPPTSPS  
 CCHPRLSLHRPALEDLLL GSEAILTCTLTGLRDASGVTF TWTPSSGKSAVQGPPDRD  
 LCGCYSVSSVLP GCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP  
 20 PSEELALNELVTLTCLARGFSPKDV LVRWLQGSQELPREKYLTWASRQEPSQGT TTF  
 AVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAE  
 VDGTCY

NT

**CTLA-4 IgAH IgA-T4 (human-oncoMLP-CTLA4EC-IgA3T1) (nucleotide sequence)**

25 atgggggtactgctcacacagaggacgtgctcagctctgctccttgactcctgtttccaagcatggcgagcatggcaatgcacgtg  
 gccagcctgctgtggtactggccagcagccgaggcatcgccagcttctgtgtgagtatgcacatccaggcaaaagccactgaggtc  
 cgggtgacagtgtctcgccaggtgacagccaggtgactgaagtctgtgcggcaacctacatgatgggggaatgagttgaccttcta  
 gatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaaggactgagggccatggacacgggactctac  
 atctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaaccagatttatgtaattgatccagaaccgt

gcccagattctgatcagccaggttcctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccc  
gactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagat  
gcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctac  
agcgtgtccagtgctcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcaactgctgcctaccccgagtccaag  
5 accccgctaaccgccaccctctcaaaatccggaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctgg  
ccctgaacgagctggtgacgtgacgtgcctggcacgtggcctcagccccaaggatgtgctggttcgctggctgcaggggtcacag  
gagctgccccgcgagaagtacgtgactgggcatcccgagcaggagccagccagggcaccaccaccttcgctgtgaccagcata  
ctgcgctggcagccgaggactggaagaagggggacaccttcctgcattggtgggccacgaggccctgccgctggccttcaca  
cagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcattggcggaggtggactgataatctaga

10 AA

**CTLA-4 IgAH IgA-T4 (amino acid sequence)**

MGVLLTQRTLSSLVLALLFPSMASMAMHVAQPAVVLAASSRGIASFVCEYASPGKAT  
EVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAM  
DTGLYICKVELMYPPYYLGIGNGTQIYVIDPEPCPDSDQPVPSTPPTPSPSTPPTSPS  
15 CCHPRLSLHRPALEDLLLSEAILTCTLTGLRDASGVFTFTWTPSSGKSAVQGPPDRD  
LCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPP  
PSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTTF  
AVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAE  
VD

20 NT

**human IgG1 CH2 with 238 mutation pro→ser (nucleotide sequence)**

cctgaactcctggggggatcgctcagtccttcttcccccaaaaccaaggacacccctcatgatctccggaccctgaggtcacat  
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa  
25 ggagtacaagtgcaaggtctccaacaagccctccagcccccatcgagaaaaccatctccaaagccaaag

AA

**human IgG1 CH2 with 238 mutation pro→ser (amino acid sequence)**

PELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA  
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK

AA

**Amino acids surrounding Pro to Ser in CH2 (amino acid sequence)**

5 PAPELLGGPS

AA

**Amino acids surrounding Pro to Ser in CH2 (amino acid sequence)**

PAPELLGGSS

NT

10 **hIgE5Bcl (nucleotide sequence)**

ggt ggt gat cac gtc tgc tcc agg gac ttc acc cc

NT

**hIgE3stop (nucleotide sequence)**

ggt ggt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g

15 NT

**hIgE3BB (leaves an open reading frame at end of gene to read into transmembrane and cytoplasmic tail domain attached at either the BamHI or SfuI sites) (nucleotide sequence)**

ggt ggt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

20 NT

**human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence)**

tgatcacgtctgctccagggaacttcaccccgccaccgtgaagatcttacagtcgtctgcgacggcgggcgggcacttcccccgac  
catccagctcctgtgcctcgtctctgggtacacccagggaactatcaacatcacctggctggaggacgggcaggtcatggacgtgg  
actgtgccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgtca  
25 gaccgcacctacactgccaggtcacctatcaagggtcacaccttgaggacagcaccaagaagtgtgcagattccaacccgagagg  
ggtgagcgcctacctaagccggcccgccggttcgacctgttcacgcaagtcgccacgatcacctgtctggtggtggacctggc

acccagcaaggggaccgtgaacctgacctggccccgggcccagtggggaagcctgtgaaccactccaccagaaaggaggagaagc  
 agcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagacctaccagtgacggg  
 tgaccacccccacctgcccagggccctcatgaggccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgtt  
 tgcgacgccggagtggccggggagccgggacaagcgcaccctcgctgcctgatccagaactcatgcctgaggacatctcggtg  
 5 cagtggtgcacaacgaggtgcagctcccgagcggcgccgacagcacgacgcagccccgcaagaccaagggtccgggttcttc  
 gtcttcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcc  
 cctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcggatccttcgaa

AA

**human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence)**

10 DHVCSRDFTPPTVKILQSSCDGGGHPPTIQLLCLVSGYTPGTINITWLEDGQVMDV  
 DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNP  
 RGVSAYLSPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEK  
 QRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYA  
 FATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGF  
 15 FVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

NT

**IFhIgGwtBcl5 (nucleotide sequence)**

ggt ggt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtc ccc agc acc (63 mer)

NT

20 **hIgGWT3xba (nucleotide sequence)**

ggt ggt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

NT

**HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant SSS)  
(nucleotide sequence)**

25 ggt ggt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt  
 gga ccg tca gtc ttc c

NT

**1D8 VH (nucleotide sequence)**

cagggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttcatta  
accagcgaatggtgtacactggattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagat  
tataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttcttaaaaatcaacagtctgcaaactga  
5 tgacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctct

AA

**1D8 VH (no leader) (amino acid sequence)**

QVQLKEAGPGLVQPTQTLSTCTVSGFSLTSDGVHWIRQPPGKGLEWMGIYYDGG  
TDYNSAIKSRLSISRDTSKSQVFLKINSLQTDDTAMYYCARIHFDYWGQGVMVTVS  
10 S

NT

**1D8 VL (no leader) (nucleotide sequence)**

gacattgtgtcactcagtcctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaa  
gttcatgtactggtaccagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatc  
15 gcttcagtggcagtggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagt  
ggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

AA

**1D8 VL (amino acid sequence)**

DIVLTQSPTTIAASPGKEKVTITCRASSSVSYMYWYQQKSGASPKLWIYDTSKLASGV  
20 PNRFSGSGSGTSYSLAINTMETEDAATYYCQQWSSTPLTFGSGTKLEIKR

NT

**1D8 scFv (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagttacatgtactggtac  
25 cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
gggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc  
tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc

aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa  
ccagcgatggtgtacactggattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagatt  
ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagtctgcaaactgat  
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcacagtcacagtcctctgatca

5 AA

**1D8 scFv (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY  
QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS  
STPLTFSGGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTVSG  
10 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS  
LQTDDTAMYYCARIHFDYWQGVMVTVSS

NT

**1D8 scFv IgG WTH WTCH2CH3 (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
15 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagtacatgtactgttac  
cagcagaagtcaggcgctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
ggctctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcggtggctcgggcgggtgggtgggtggcgccggtatc  
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa  
20 ccagcgatggtgtacactggattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagatt  
ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaatcaacagtctgcaaactgat  
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcacagtcacagtcctctgatcaggagcccaa  
atcttgtgacaaaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttctcttccccccaaaacc  
aaggacacctcatgatctcccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttcaa  
25 ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag  
cgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcg  
agaaaacaatctcaaagccaaaggcgagccccgagaaccacaggtgtacacctgcccccatcccggtgatgactgaccaaga  
accaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccggagaa  
caactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggc

agcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagccctctccctgtctccggga  
aatgatctaga

AA

**1D8 scFv IgG WTH WTCH2CH3 (amino acid sequence)**

5 MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY  
QQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS  
STPLTFGSGTKLEIKRGGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG  
FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAISKRLSISRDTSKSQVFLKINS  
LQTDDTAMYYCARIHFDYWGGQVMVTVSSDQEPKSCDKTHTCPPCPAPPELLGGPS  
10 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ  
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS  
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

15 **1D8 scFv IgG MTH MTCH2CH3-CD80 (nucleotide sequence)**

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac  
cagcagaagtcaggcgctccctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
20 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcggtggtgggtcgggtggcggcggtatc  
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa  
ccagcgatggtgtactggattcgacagcctccaggaaaggtctggaatggatgggaataatatattatgatggaggcacagatt  
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaatcaacagctctgcaaaactgat  
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa  
25 atcttctgacaaaactcacacaagcccaccgagcccagcacctgaactcctggggggatcgtcagttctctctccccccaaaaccc  
aaggacacctcatgatctccggaccttgaggtcacatgcgtgggtgggtggacgtgagccacgaagacctgagggtcaagttcaa  
ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag  
cgtctcaccgtctctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcg  
agaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtgatgactgaccaaga

accaggtcagcctgacctgcctggcctcaaggcttctatcccagcgacatcgccgtggagtgaggagagcaatgggcagccggagaa  
 caactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggc  
 agcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggta  
 aagcggatccttcgaacctgctccatcctgggccattacctaatactcagtaaatggaatgttgatgctgcctgacctactgctt  
 5 gccccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

AA

**1D8 scFv IgG MTH MTCH2CH3-CD80 (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY  
 QQKSGASPKLWIYDTSKLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS  
 10 STPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG  
 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS  
 LQTDDTAMYYCARIHFDYWQGVMVTVSSDLEPKSSDKTHTSPSPAPELLGGSSV  
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY  
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
 15 PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSK  
 LTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKADPSNLLPSWAITLISVN  
 GIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**1D8 scFv IgG WTH WTCH2CH3-CD80 (nucleotide sequence)**

20 aagcttatggatttcaagtcagatttctcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagttacatgtactggtag  
 cagcagaagtcaggcgctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgtagtagtactccgc  
 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtgggtggcggcgatctc  
 25 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaaatcaacagctgcgaactgat  
 gacacagccatgtattactgtccagaatccacttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa  
 atcttgtagaaaaactcacatgccaccgtgccagcacctgaactcctggggggaccgtcagttctcttccccccaaaaccc



aaggacacccctcatgatctccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaa  
 ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcag  
 cgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcg  
 agaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacccctgccccatcccgggatgagctgaccaaga  
 5 accaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa  
 caactacaagaccacgcctcccgtgtggtgactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggc  
 agcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggta  
 aagcggatccttgaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctt  
 gccccaatgagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

10 AA

**1D8 scFv IgG WTH WTCH2CH3-CD80 (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGKEKVTITCRASSSVSYMYWY  
 QQKSGASPKLWIYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS  
 STPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG  
 15 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS  
 LQTDDTAMYYCARIHFDYWQGVMVTVSSDLEPKSCDKTHTCPPCPAPELLGGPS  
 VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ  
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
 PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYS  
 20 KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKADPSNLLPSWAITLISV  
 NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**Anti-human CD3 scFv WTH WTCH2CH3 (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac  
 25 agactacatcctcctgtctgcctctctgggagacagagtcaccatcagttgcagggaagtcaggacattcgcaattatttaaactgg  
 taticagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcctcatcaaggttcagtggcag  
 tgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttactttgccaacagggtataacgcttccg  
 tggacgttcggtaggagccacaaactggtaaccaaaccgggagctcggtaggcggtggctcgggcggtggtaggcggtggcg  
 cggatctatcgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggcctctggttac

tcattcactggctacatcgtgaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccatacaaaaggtctt  
actacctacaaccagaaattcaagggcaaggccacattaactgtagacaagtcacagcagcctacatggagctcctcagctcg  
acatctgaagactctgcagtctattactgtgcaagatctgggtactatggtgactcggactgggtacttcgatgtctggggcgagggac  
cacggtcaccgtctcctctgatcaggagcccaaatcttgacaaaactcacacatgccaccgtgccagcacctgaactcctggg  
5 gggaccgtcagttctctcttcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtgga  
cgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagccgcggga  
ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgc  
aaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtac  
accctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatcg  
10 cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctcctct  
acagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca  
ctacacgcagaagagcctctccctgtctccgggtaaatgatctaga

**Anti-human CD3 scFv WTH WTCH2CH3 (amino acid sequence)**

25 **2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3** (2h7-40.2.220Ig + restriction sites)  
(nucleotide sequence)

gtgggtctgggacctctactctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggttttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaaggtggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcaactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
5 tctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaatccaactctgaagaagcaaaagaggaggccaaaaaggaggaaagcaagaatctaacagc  
gtcgacattgttctgactcagctccagccaccctgtctgtgactccaggagatagagtctctcttctgcagggccagccagagtatt  
agcgactacttacactggtatcaaaaaatcacatgagtctccaaggcttctcatcaaatatgcttccattccatctctgggatcccct  
10 ccagggtcagtggcagtgatcagggtcagatttactctcagtatcaacagtgtggaacctgaagatgttggaatttattactgtcaac  
atggtcacagctttccgtggacgttcgggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggcggaggtgggtc  
gggtggcggcgatctcagatccagttggtgcaatctggacctgagctgaagaagcctggagagacagtcaggatctcctgcaag  
gcttctgggtatgccttcacaactactggaatgcagtgggtgcaagagatgccaggaaagggttggaatggattggctggataaac  
acccactctggagtgcacaaatatgtagaagactcaaggacggttgccttctcttggaaacctctgccaacactgcatatttacag  
15 ataagcaacctcaaagatgaggacacggctacgtatttctgtgtgagatccgggaatggttaactatgacctggcctactttgcttactg  
gggccaagggacactggtcactgtctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctga  
actcctggggggatcgtcagttctcttccccccaaaaccaaggacacctcatgatctccggaccttgaggtcacatgcgtg  
gtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagc  
cgcgaggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagta  
20 caagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccaca  
ggtgtacacctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaaggcttctatcccagcg  
acatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcctt  
cttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgca  
caaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

25 AA

**2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3 (2H7-40.2.220Ig) (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN

PPTFGAGTKLELKGGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQSNSEEAKKEEAK  
 KEEAKKSNSVDIVLTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSHESPRLLI  
 5 KYASHSISGIPSRFSGSGSGSDFTLSINSVEPEDVGIYYCQHGHSFPWTFGGGTKLEIK  
 RGGGGSGGGGSGGGGSGGGGSIQLVQSGPELKKPGETVRISCKASGYAFTTTGMQWVQE  
 MPGKGLKWIGWINTPLWSAKICRRLQGRFAFSLETSANTAYLQISNLKDEDTATYF  
 CVRSGNGNYDLAYFAYWGQGTLLTVSDQEPKSSDKTHTSPSPAPELLGGSSVFLF  
 PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS  
 10 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS  
 RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT  
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

**5B9 VH (includes the VH leader peptide) (nucleotide sequence)**

15 atggctgtcttgggctgctcttgccttggtgacattccaagctgtgtcctatcccaggtgcagctgaagcagtcaggacctggcct  
 agtcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcattaactacatgtgttacactgggttcgccagtctcca  
 ggaaagggtctggagtggctgggagtgatatggagtgggtggaatcacagactataatgcagctttcatatccagactgagcatcacc  
 aaggacgattccaagagccaagttttcttaaaatgaacagtctgcaacctaatacacagccatttattactgtgccagaaatgggggt  
 gataactacccttattactatgctatggactactgggggtcaaggaacctcagtcaccgtctcctca

20 NT

**5B9 VH (minus the leader) (nucleotide sequence)**

caggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtctctggtttctcattaac  
 tacctatgtgttacactgggttcgccagtctccagaaagggtctggagtggctgggagtgatatggagtgggtggaatcacagactat  
 aatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttcttaaaatgaacagtctgcaacctaatac  
 25 acagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaaggaacctcagtcacc  
 gtctcctca

AA

**5B9 VH (includes leader peptide) (amino acid sequence)**

MAVLGLLFCLVTFPSCVLSQVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVR  
QSPGKGLEWLGVWSSGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDAIYYC  
ARNGGDNYPYYYAMDYWGQGTSVTVSS

5 **5B9 VH** (no leader peptide) (amino acid sequence)

QVQLKQSGPGLVQSSQSLSITCTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSSGI  
TDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDAIYYCARNGGDNYPYYYAMDY  
WGQGTSVTVSS

NT

10 **5B9 VL** (nucleotide sequence)

atgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctcca  
atccagtcactcttgaacatcagcttccatctcctgcaggctagtaagagtctcctacatagtaatggcatcacttattgtattggtatc  
tgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagtccagacaggttcagtagcagtg  
gtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgttattactgtgctcaaaatctagaactccgct  
cacgttcggtgctgggaccaagctggagctgaaacgg

AA

**5B9 VL** (amino acid sequence)

MRFSAQLLGLLVLPWGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSNGITYL  
YWYLQKPGQSPQLLIYQMSNLA SGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA  
20 QNLELPLTFGAGTKLELKR

NT

**5B9 scFv** (nucleotide sequence)

aagcttgccgcatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcag  
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggctagtaagagtctcctacatagtaatggcatcactta  
25 ttgtattggtatctgcagaagccaggccagctcctcagctcctgattatcagatgtccaacctgcctcaggagtccagacaggttc  
agtagcagtggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgttattactgtgctcaaaatct  
agaactccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggctcgggcgggtgggtcgggtg  
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagacctgtccatcacctgcacagctt

ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggctctggagtggctgggagtgatatggagtgg  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctttaaataaacagt  
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa  
ggaacctcagtcaccgtctctct

5 AA

**5B9 scFv (amino acid sequence)**

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSNGITYL  
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA  
QNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT  
10 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV  
FFKMNSLQPNDTAIYYCARNGGDNPYYYAMDYWGQGSVTVSS

NT

**5B9 scFv-hmtIgG1-hCD80 (nucleotide sequence)**

aagcttgccgcatgaggttctctgctcagcttctggggctgctgtgctctggatccctggatccactgcagatattgtgatgacgcag  
15 gctgcattctccaatccagtcactcttgaacatcagcttccatctctgcaggtctagtaagagtctctacatagtaatggcatcactta  
tttgattggtatctgcagaagccaggccagctctctcagctcctgatttatcagatgtccaaccttgccctaggagctccagacaggttc  
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgttttattactgtgctcaaaatct  
agaacttccgtcacgttcgggtgctgggaccaagctggagctgaaacgggggtggcggtggctcgggcgggtgggtgggtcgggtgg  
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagctct  
20 ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggctctggagtggctgggagtgatatggagtgg  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctttaaataaacagt  
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa  
ggaacctcagtcaccgtctctctgatctggagcccaaatcttctgacaaaactcacacaagcccaccgagcccagcacctgaactc  
ctggggggatcgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggacctgaggtcacatgcgtggtg  
25 gtggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgc  
gggaggagcagtacaacagcacgtacctgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa  
gtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt  
gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgaca  
tcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcctctt

cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca  
 accactacacgcagaagagcctctccctgtctccggtaaagcggatccttgaacctgctcccatcctgggccattacctaatactca  
 gtaaatggaattttgtgatatgctgcctgacctactgctttgcccccaagatgcagagagagaaggaggaatgagagattgagaaggg  
 aaagtgtacgccctgtataaatcgataactcgag

5

AA

**5B9 scFv-hmtIgG1-hCD80 (amino acid sequence)**

MRFSAQLLGLLVLPWGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSGITYL  
 YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA  
 10 QNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT  
 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV  
 FFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGSVTVSSDLEPKSSDKTH  
 TSPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG  
 VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS  
 15 KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKAD  
 PSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

20 NT

**2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (nucleotide sequence)**

aagcttatggatttcaagtcagatcttcagcttctgctaatacagtcgcttcagtcataatgtccagaggagtcgacattgtgtcaccca  
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
 atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcaccaacgtagaatctggggtccctgccagg  
 25 tttagtggcagtggtctgggacagactcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
 aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt  
 gggcgcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
 cagggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaatgaa

cagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatggtatggactactgggggtcaa  
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttgacaaaactcacacatgccaccgtgccagcacctgaactc  
 ctgggggggaccgtcagtcctcctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgctggtg  
 gtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc  
 5 gggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa  
 gtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt  
 gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtcaaaggcttctatcccagcgaca  
 tcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgctgctggactccgacggctccttctt  
 cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcaca  
 10 accactacacgcagaagagcctctccctgtctccgggtaaagcggatccttcgaacctgctcccatcctgggccattaccttaattc  
 gtaaatggaattttgtgatatgctgcctgacctactgcttggcccaagatgcagagagagaaggaggaatgagagattgagaagg  
 aaagtgtacgccctgtataaatcgat

AA

**2e12 scFv WTH CH2 CH3 (2e12 scFv-WthIgG-CD80) (amino acid sequence)**

15 MDFQVQIFSFLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL  
 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ  
 QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLITC  
 TVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ  
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWGQGSVTVSSDLEPKSCDKT  
 20 HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
 GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKA  
 DPSNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

25 NT

**2H7-human IgE Fc (CH2-CH3-CH4) (nucleotide sequence)**

aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatcagtgcttcagtcataattgccagaggacaaattgttctctc  
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcaccaatgacttgaggccagctcaagtgttaagttacatgcactgg  
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga



gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggttttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagggtggcgggtggctcgggcgggtggtgatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat  
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5 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctctgatcacgtctgctccagggacttcaccccgccaccgtgaagatcttacagtcgtcctgcgacggcggcgggca  
cttcccccgaccatccagctcctgtgcctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggt  
catggagctggacttgccaccgcctctaccacgcaggaggggtgagctggcctccacacaaagcgagctcacctcagccagaag  
10 cactggctgtcagaccgcactacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattcc  
aaccgagaggggtgagcgctacctaagccggcccagcccgttcgacctgttcacccgaagtcgccacgatcacctgtctggt  
ggtggacctggcaccagcaaggggaccgtgaacctgacctgggtccgggaccagtggaagcctgtgaaccactccaccagaaa  
ggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgccgggtgggcacccgagactggatcgagggggagacct  
accagtgcagggtgaccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcgccccgcgtgctgccccg  
15 gaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcacccctgcctgcctgatccagaacttcagcctg  
aggacatctcgggtgcagtggctgcacaacgaggtgcagctcccgacggcgacagcacgacgcagccccgaagaccaag  
gggtccggcttctctgcttccagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcac  
gaggcagcgagccccctacagaccgtccagcgagcgggtgtctgtaaatcccgtaaatgataatctaga

AA

20 **2H7 scFv IgE (CH2-CH3-CH4) (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
25 QLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSDHVCSDRFTPTVKIL  
QSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELAST  
QSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSPSPFDLFI  
RKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGT  
RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLA

CLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQK  
DEFICRAVHEAASPSQTVQRAVSVNPGK

NT

**2H7 scFv MH (SSS) MCH2WTCH3 (nucleotide sequence)**

5 aagcttgccgcatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
cccccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctggcggtggtggatctggaggaggtgggagc  
10 tctcaggcttatctacagcagctctggggctgagctggtagggcctggggcctcagtgaaagatgctcgtcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact  
tcctacaatcagaagtcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga  
15 tcgtcagttcttcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga  
20 gtgggagagcaatgggcagccggagaaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg  
cagaagagccctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv MH (SSS) MCH2WTCH3 (amino acid sequence)**

25 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFVDVWGTGTTVTVSSDQEPKSSDKTHTSPP

SPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP  
VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

5 NT

**5B9 scFv MTHWTCH2CH3 (nucleotide sequence)**

aagcttgccgccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcag  
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta  
tttgattggtatctgcagaagccaggccagctctcctcagctcctgattatcagatgtccaacctgcctcaggagtcccagacaggttc  
10 agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct  
agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtgggtgggtcgggtgg  
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtct  
ctggtttctcattaactacctatgctgtacactgggttcgccagctctccaggaaagggtctggagtggctgggagtgatatggagtgg  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt  
15 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggtcaa  
ggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc  
tggggggaccgtcagcttctccttcccccaaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgg  
tggacgtgagccacgaagacctgaggtcaagtcaactgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
ggaggagcagtaaacagcacgtaccgtgtgtgtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaag  
20 tgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgt  
acacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

25

AA

**5B9 scFv MTHWTCH2CH3 (amino acid sequence)**

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSHNGITYL  
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA  
QNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT  
5 CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV  
FFKMNSLQPNDTAIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSSDQEPKSSDKT  
HTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
10 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

**Human IgG1 hinge mutations**

NT

**2H7 scFv- MTH (CSS) WTCH2CH3 (nucleotide sequence)**

Nucleotide:

15 aagcttgccgccatggattttcaagtcagattttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
gtgggtctgggacctctactcttcacaatcagcagagtgaggagctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
20 tctcaggcttatctacagcagctctggggtgagctggtaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
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AA

5 **2H7 scFv- MTH (CSS) WTCH2CH3 (amino acid sequence):**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
10 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTSPP  
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPP  
VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

15 NT

**2H7 scFv- MTH (SCS) WTCH2CH3 (nucleotide sequence)**

aagcttgccgccatggattttcaagtcagatttcagcttctgctaatacagtcgttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcattctccaggggagaaggtcaccaatgacttgaggccagctcaagtgtaagtacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtctgcttcagtgga  
20 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
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25 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacaggaccac  
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cagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggt

ctccaacaaagccctcccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct  
 gcccccatcccggtgatgctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatcccagcgacatcgccgtgg  
 agtgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctacagc  
 aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
 5 gcagaagagccctcctctgtctccgggtaaatgatctaga

AA

**2H7 scFv- MTH (SCS) WTCH2CH3 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
 QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
 10 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTCPP  
 SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
 15 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP  
 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

NT

**2H7 scFv- MTH (SSC) WTCH2CH3 (nucleotide sequence)**

aagcttcccgccatggattttcaagtgcagatttcagcttctgctaatacgtgcttcagtcataattgccagaggacaaattgttctctc  
 20 ccagcttccagcaatctgtctgcattctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggatcctcccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
 25 ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctgcggtctatttctgtgaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtgccagcacctgaactcctggggggg  
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agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggag  
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5 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctctctacagc  
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
gcagaagagcctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv- MTH (SSC) WTCH2CH3 (amino acid sequence)**

10 MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP  
15 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP  
VLDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

20 **HIgGMHcys1 (nucleotide sequence)**

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

NT

**HIgGMHcys2 (nucleotide sequence)**

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct cca ccg tgc

25 NT

**HIgGMHcys3 (nucleotide sequence)**

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt cca ccg tcc cca gca cct

NT

**HuIgG1 MTCH3Y405 (nucleotide sequence)**

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctg  
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc  
5 gtgctggactccgacggctccttctacatctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgc  
tccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA

**HuIgG1 MTCH3Y405 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
10 VLSDGSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

**HuIgG1 MTCH3A405 (nucleotide sequence)**

gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctg  
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc  
15 gtgctggactccgacggctccttcgccctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg  
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA

**HuIgG1 MTCH3A405 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
20 VLSDGSFALYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

**HuIgG1 MTCH3A407 (nucleotide sequence)**

Gggcagccccgagaaccacaggtgtacaccctgccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcct  
gggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcc  
25 cgtgctggactccgacggctccttcttctcgcagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat  
gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

AA



**HuIgG1 MTCH3A407 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
VLDS DGSFFLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

5 **HuIgG1 MTCH3Y405A407 (nucleotide sequence)**

gggcagccccgagaaccacaggtgtacacctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctg  
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc  
gtgtggactccgacggctccttctacctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg  
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

10 AA

**HuIgG1 MTCH3Y405A407 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
VLDS DGSFY LASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

15 **HuIgG1 MTCH3A405A407 (nucleotide sequence)**

gggcagccccgagaaccacaggtgtacacctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctg  
gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctccc  
gtgtggactccgacggctccttcgccctcgccagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg  
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgggtaaatga

20 AA

**HuIgG1 MTCH3A405A407 (amino acid sequence)**

GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
VLDS DGSFALASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

25 **2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (nucleotide sequence)**

aagcttgccgccatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagtacatgactgg

taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
 gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacat  
 5 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
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 10 gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
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 tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
 ccccatcccgaggagatgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtgg  
 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctgctggactccgacggctccttctacctctatagc  
 15 aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
 gcagaagagcctctccctgtccccgggtaaatgatctaga

AA

**2H7 scFv MTH (SSS) WTCH2MTCH3Y405 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
 20 QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP  
 SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 25 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
 KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP  
 PVLDSGDSFYLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

**2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence)**

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5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc  
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15 ccccatccccgggaggagatgaccaagaaccaggtcagcctgacctggtcaaaggcttctatccagcgacatcgccgtgg  
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caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactaca  
cgcagaagagcctctccctgtccccgggtaaatga

AA

20 **2H7 scFv MTH (SSS) WTCH2MTCH3A405 (nucleotide sequence)**

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
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gytftsynmhwwkqtrprglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy  
fdvwgtgtvtvssdqepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv  
25 evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsreemtknqvslt  
clvkgfypsdiavewesngqpennykttppvldsdgsfalyskltdksrwqqgnvfscsvmhealhnhytqkslslspgk

NT

**2H7 scFv MTH (SSS) WTCH2MTCH3A407 (nucleotide sequence)**

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 taccagcagaagccaggatcctcccccaccctggatttatgcccacccaacctggcttctggagtcctgctcgcttcagtgga  
 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
 5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctggcggtggtggatctggaggaggtgggagc  
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 10 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga  
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 gccacgaagaccctgaggtcaagttaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
 agtacaacagcagctaccgtgtggtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggct  
 tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg  
 15 ccccatccccgggaggagatgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatgccgtgg  
 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcctcgcag  
 caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactaca  
 cgcagaagagcctctccctgtccccgggtaaatga

AA

20 **2H7 scFv MTH (SSS) WTCH2MTCH3A407 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
 QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 25 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP  
 SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
 KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP  
 PVLDSGDSFFLASKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK

NT

**2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (nucleotide sequence)**

aagcttgccgcatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
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5 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga  
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10 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
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gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
15 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggct  
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caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactaca  
20 cgcagaagagcctctccctgtccccgggtaaatga

AA

**2H7 scFv MTH (SSS) WTCH2MTCH3Y405A407 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
25 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVYYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSP  
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP  
PVLDSGDSFYLASKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

NT

**2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (nucleotide sequence)**

5 aagcttgccgccatggattttcaagtcagattttcagcttctgctaatacagtcgttcagtcataattgccagaggacaaattgttctctc  
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taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
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15 ccgtcagttctcttctccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgggtgggtgacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggct  
tccaacaaagccctcccagcccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
ccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtgg  
20 agtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcgcctcgcga  
gcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactac  
acgcagaagagcctctccctgtccccgggtaaatga

AA

**2H7 scFv MTH (SSS) WTCH2MTCH3A405A407 (amino acid sequence)**

25 MDFVQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSPP

SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP  
PVLDSDSGSFALASKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK

5 NT

**2H7 scFv MTH (SCC) WTCH2CH3 (nucleotide sequence)**

aagcttgccgccatggattttcaagtcagattttcagcttcctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
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taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
10 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggatttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggtcttggtacacat  
ttaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tcctacaatcagaagtcaagggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
15 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
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agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggag  
cagtacaacagcagctaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggt  
20 ctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacct  
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agtgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagc  
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
gcagaagagcctctccctgtctccggtaaatgatctaga

25 AA

**2H7 scFv MTH (SCC) WTCH2CH3 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG

YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTCPP  
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
 5 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP  
 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

NT

**2H7 scFv MTH (CSC) WTCH2CH3 (nucleotide sequence)**

aagcttgccgccatggattttcaägtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
 10 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga  
 gtgggtctgggacctctactcttcacaatcagcagagtggaggctgaagatgctgccacttactgccagcagtgagggtttaac  
 ccaccacgttcgggtctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtgatctggaggaggtgggagc  
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 15 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
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 ggtcaccgtctctctgatcaggagcccaatctgtgacaaaactcacacatctccaccgtgccagcacctgaactcctgggggga  
 ccgtcagctctctcttccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtga  
 20 gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
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 cccccatccgggatgagctgaccaagaaccaggtcagcctgacctggtcaaaggcttctatccagcgacatcgccgtgga  
 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttcttctctacagca  
 25 agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacag  
 cagaagagcctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv MTH (CSC) WTCH2CH3 (amino acid sequence)**



MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHYQ  
 QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
 PPTFGAGTKLELKDGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 5 QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTSPP  
 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
 HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPP  
 VLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

10 NT

**2H7 scFv MTH (CCS) WTCH2CH3 (nucleotide sequence)**

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 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtctgcttcagtgga  
 15 gtgggtctgggaccttactctctcacatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
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 ccgtcagctctctctccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga  
 gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc  
 25 tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
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 agctcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcatgaggctctgcacaaccactacacg  
 cagaagagcctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv MTH (CCS) WTCH2CH3 (amino acid sequence)**

MDFQVQIFSFLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
5 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWYFDVWGTGTTVTVSSDQEPKSCDKTHTCPP  
SPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
10 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP  
VLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

**HuIgAHIgA-T4-ORF (nucleotide sequence)**

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact  
15 gcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg  
tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca  
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cagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgccgctggccttcacacagaagacca  
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AA

**HuIgAHIgA-T4-ORF (amino acid sequence)**

25 DQVPVSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVT  
FTWTPSSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL  
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP  
REKYLTWASRQEPSQGTTTFAVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKT  
IDRLAGKPTHVNVSVVMAEVDADPSN

NT

**HuIgAHIgA-T4-ORF (nucleotide sequence)**

tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcact  
gcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtg  
5 tcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctgctacagcgtgtcca  
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accgccaccctctcaaaatccggaacacattccggcccagggtccacctgctgccgccgccgtcggaggagctggccctgaacg  
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cgcgagaagtacgtgacttgggcatcccgccaggagcccgccagggcaccaccaccttcgctgtgaccagcatactgcgcgtgg  
10 cagccgaggactggaagaagggggacaccttctcctgcatggtgggcccacgaggccctgccgctggccttcacacagaagacca  
tcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcacggcgagggtggacgcggatccttgaac

AA

**HuIgAHIgA-T4-ORF (amino acid sequence)**

DQPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLGSEAILTCTLTGLRDASGVT  
15 FTWTPSSGKSAVQGPDRDLGCGYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPL  
TATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELP  
REKYLTWASRQEPSQGTTFVAVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKT  
IDRLAGKPTHVNVSVVMAEVDADPSN

NT

20 **1D8-IgAH IgA-T4-CD80 (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
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cagcagaagtgcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagt  
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ctcaactccacctacccccatctccctcaactccacctacccccatctccctcatgctgccacccccgactgtcactgcaccgaccggcc  
 ctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctgga  
 cgccctcaagtgggaagagcgctgttcaaggaccacctgacctgtacctctgtggctgctacagcgtgtccagtgtcctgccgggct  
 gtgccgagccatggaacctgggaagaccttcactgactgctgcctaccccgagtccaagaccccgtaaccgccaccctctca  
 5 aaatccggaacacattccggccccgaggtccacctgctgccgccgcccgtcgaggagctggccctgaacgagctggtgacgctg  
 acgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacct  
 gactgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcactgctgcgctggcagccgaggactg  
 gaagaagggggacaccttctctgcatggtggggccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcg  
 ggtaaacccacccatgtcaatgtgtctgttgcacggcgaggtggacgcgatcctcgaacaacctgctcccatctggggccatta  
 10 ccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctttgccccaatgagagagagaaggaggaatgagaga  
 ttgagaagggaagtgtacgcctgtataaatcgatac

AA

**1D8 scFv IgAH IgA-T4-CD80 (amino acid sequence)**

MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY  
 15 QQKSGASPKLWIYDTSKLASGVNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS  
 STPLTFSGGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSLTCTVSG  
 FSLTSDGVHWIRQPPGKGLEWMGIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS  
 LQTTDDTAMYYCARIHFDYWQGVMVTVSSDQVPSTPPTPSPSTPPTPSPSCCHPRL  
 SLHRPALEDLLLSEAILTCTLTGLRDASGVTFWTWTPSSGKSAVQGPDRDLGCYS  
 20 VSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPPPSEELAL  
 NELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILR  
 VAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEVDADPS  
 NNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

NT

**25 human IgE Fc (CH2-CH3-CH4) ORF (nucleotide sequence)**

tgatcacgtctgtccagggacttcaccccgcccaccgtgaagatcttacagtcgtcctgcgacggcgggcgggcacttcccccgac  
 catccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtgg  
 acttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgtca  
 gaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattccaaccgagagg

ggtgagcgcctacctaagccggcccagcccgttcgacctgttcacccgaagtcgcccacgatcacctgtctggtggtggacctggc  
 acccagcaaggggaccgtgaacctgacctggccccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagc  
 agcgcaatggcacgttaaccgtcacgtccaccctgccgggtgggcacccgagactggatcgagggggagacctaccagtgcaggg  
 tgaccacccccacctgcccagggccctcatgcggtccacgaccaagaccagcgccccgcgtgctgccccggaagtctatgcgtt  
 5 tgcgacgccggagtggccggggagccgggacaagcgcaccctcgctgcctgatccagaacttcatgcctgaggacatctcggtg  
 cagtggctgcacaacgaggtgcagctcccgagccccggcacagcacgacgcagccccgaagaccaagggtccgggtcttc  
 gtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagcc  
 cctcacagaccgtccagcgagcgggtgtctgtaaatacccggtaaagcggtaccttcgaa

AA

10 **human IgE Fc (CH2-CH3-CH4) ORF (amino acid sequence)**

DHVCSDFTPTVKILQSSCDGGGHPPTIQLLCLVSGYTPGTINITWLEDGQVMDV  
 DLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNP  
 RGVSAYLSPSPFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEK  
 QRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYA  
 15 FATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGF  
 FVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPS

NT

**1D8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
 20 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagttacatgtactggtac  
 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgtagtactccgc  
 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtggcggcgatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa  
 25 ccagcgatggtgtactggttcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagtttcttaaaaatcaacagctctgcaaaactgat  
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctgatcacgtctgctcc  
 agggacttcaccccgccaccgtgaagatcttacagtcgtcctgcgacggcgggcacttccccccgacatccagctcctgtg  
 cctcgtctctgggtacaccccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtggacttgcaccgcctc

taccacgcaggagggtgagctggcctccacacaaagcgagctcaccctcagccagaagcactggctgtcagaccgcactacac  
 ctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaagtgtgcagattccaacccgagaggggtgagcgcctacc  
 taagccggcccagcccgttcgacctgttcacgcaagtcgccacgatcacctgtctggtgggtggacctggcacccagcaagggg  
 accgtgaacctgacctggccccggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcaatggcac  
 5 gttaacctgcacgtccaccctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtgacctacccccca  
 cctgccaggggccctcatcggtccacgaccaagaccagcgccccgcgtgctgccccggaagtctatgcgtttgcgacgccgga  
 gtggccggggagccgggacaagcgcaccctcgctgcctgatccagaactcatgcctgaggacatctcggtgcagtggctgcac  
 aacgaggtgcagctcccgagcggccggcacagcacgacgcagccccgcaagaccaagggtccggcttctctcagccgc  
 ctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtccatgaggcagcgagccccctcacagacc  
 10 gtccagcgagcgggtgtctgtaaaccggtaaagcggatccttcgaagctcccatcctgggccattaccttaatctcagtaaatggaat  
 tttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacg  
 ccctgtataaatcgata

AA

**1D8-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)**

15 MDFQVQIFSLLISASVIMSRGVDIVLTQSPTTIAASPGEKVTITCRASSSVSYMYWY  
 QKSGASPKLWIYDTSKSLASGVPNRFSGSGSGTSYSLAINTMETEDAATYYCQQWS  
 STPLTFGSGTKLEIKRGGGSGGGGSGGGGSQVQLKEAGPGLVQPTQTLSTCTVSG  
 FSLTSDGVHWIRQPPGKGLEWMGIIYYDGGTDYNSAIKSRLSISRDTSKSQVFLKINS  
 LQTTDDTAMYYCARIHFDYWGQGVMVTVSSDHVCSRDFTPPTVKILQSSCDGGGHF  
 20 PPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLQKH  
 WLSDRITYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRSPFDLFIKRSPTITCLV  
 VDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGTRDWIEGETY  
 QCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMP  
 DISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVH  
 25 EAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCLTYCFAPRCRERRRN  
 ERLRRESVRPV

NT

**5B9-IgAH IgA-T4-CD80 (nucleotide sequence)**

aagcttgccgcatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcag  
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctctacatagtaatggcatcactta  
tttgattggtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaaccttgccctcaggagtcacagaggttc  
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtgagggtgaggatgtgggtgtttattactgtgctcaaaatct  
5 agaacttccgctcacgttcgggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggtcgggtgg  
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtct  
ctggtttctcattaactacatgctgtacactgggttcgccagctctccaggaaagggtctggagtggctgggagtgatatggagtgggt  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagttttctttaaataaacagat  
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactggggtcaa  
10 ggaacctcagtcaccgtctcctctgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctca  
tgctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgacc  
ggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacct  
ctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgcactgctgcctac  
cccagtgccaagaccccgctaaccgccacctctcaaaatccggaacacattccggcccagggtccacctgtgccgccgccgt  
15 cggaggagctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggct  
gcaggggtcacaggagctgccccgcgagaagtacgtgacttgggcatcccgagcaggagcccagccagggcaccaccaccttcg  
ctgtgaccagcactactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggccacgaggccctgc  
cgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccaccatgtcaatgtgtctgttgcagggcggaggtggacg  
cggatccttcgaacaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgctgacctactgctt  
20 gccccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

AA

**5B9-IgAH IgA-T4-CD80 (amino acid sequence)**

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSHNGITYL  
YWYLQKPGQSPQLLIYQMSNLSGVPDRFSSSGSGTDFTLRISRVEADVGVYYCA  
25 QNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT  
CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV  
FFKMNSLQPNDAIYYCARNGGDNPYYYAMDYWGQGSVTVSSDQVPVPSTPPTP  
SPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWPSSGKS  
AVQGPPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNT

FRPEVHLLPPPSEELALNELVTLTCLARGFSPKDLVRWLQGSQELPREKYLTWASR  
QEPSQGTTTTFAVTSILRVA AEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTH  
VNVSVVMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRR  
ESVRPV

5 NT

**5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)**

aagcttgccgcatgaggttctctgctcagcttctggggctgctgtgctctggatccctggatccactgcagatattgtgatgacgcag  
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta  
tttgattggtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtccagacaggttc  
10 agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct  
agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcgggcggtgggtgggtcgggtgg  
cggcggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtct  
ctggtttctcattaactacatgctgtacactgggttcgccagctcctcaggaaagggctgagtggtgggtgagtgatggagtgg  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt  
15 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactaccttattactatgctatggactactggggtaa  
ggaacctcagtcaccgtctcctctgatcacgtctgctccaggacttcacccgccaccgtgaagatcttacagtcgtcctgcgacg  
gcggcgggcacttcccccgacctccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggag  
gacgggcaggtcatggagctggactgtccaccgccttaccacgcaggagggtgagctggcctccacacaaagcgagctcaccc  
tcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaagggtcacaccttgaggacagcaccaagaag  
20 tgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgaagtcgccacgat  
cacctgtctggtgggtggacctggcaccagcaaggggaccgtgaacctgacctgggtcccgggcccagtggaagcctgtgaaccac  
tccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgcccgtgggcacccgagactggatcgag  
ggggagacctaccagtgcagggtgacctacccccacctgccaggggccctcatgcggtccacgaccaagaccagcggccgcg  
tgctgccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgaccctcgcctgcctgatccagaac  
25 ttcatgcctgaggacatctcgggtgcagtgggtgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgc  
aagaccaagggtccggcttctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccg  
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catcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgcttgccccaagatgcagagagagaag  
gaggaatgagagattgagaagggaagtgtagccctgtataaatcgata



AA

**5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)**

MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLLSHNGITYL  
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA  
5 QNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT  
CTVSGFSLTTYAVHWVRQSPGKGLEWLGVWSGGITDYNAAFISRLSITKDDSKSQV  
FFKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGTSVTVSSDHVCSRDFTPP  
TVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEG  
ELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAAYLSRPS  
10 PFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTS  
TLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSR  
DKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRA  
EWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCL  
TYCFAPRCRERRRNERLRRESVRPV

15 NT

**2e12-scFv-IgAH IgA-T4-CD80 (nucleotide sequence)**

aagcttatggatttcaagtcagatttccagcttctgctaatacagtcgttcagtcataatgtccagaggagtcgacattgtgtcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctcgagagccagtgaaagtggtgaatattatgcacaagttta  
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtcctgccagg  
20 tttagtggcagtggtctgggacagactcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt  
ggcggcgggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct  
caggggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaaactccaagagccaagtttcttaaaaatgaa  
25 cagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctca  
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ggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacct  
ctgtggctgctacagcgtgtccagtgctctgccgggctgtgccgagccatggaacctgggaagacctcactgtcactgtgcctac

cccgagtccaagacccccgtaaccgccaccctctcaaaatccggaaacacattccggcccagggtccacctgctgccgccgccgt  
 cggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggct  
 gcaggggtcacaggagctgccccgcgagaagtacctgacttgggcatcccgagggagcccagggcaccaccaccttcg  
 ctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatgggtgggccacgaggccctgc  
 5 cgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcaggggaggtggacg  
 cggatccttcgaacaacctgctcccatcctgggccattaccttaatctcagtaaattggaattttgtgatagctgcctgacctactgctt  
 gccccaatgagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

AA

**2e12-scFv-IgAH IgA-T4-CD80 (amino acid sequence)**

10 MDFQVQIFSLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL  
 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ  
 QSRKVPWTFGGGTKLEIKRGGGGSGGGGSGGGGSQVQLKESGPGLVAPSQSLITC  
 TVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ  
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWQGTSVTVSSDQVPSTPPTP  
 15 SPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEAILTCTLTGLRDASGVTFWTWTPSSGKS  
 AVQGPPDRDLGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNT  
 FRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASR  
 QEPSQGTTTTFAVTSILRVAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTH  
 VNVSVMMAEVDADPSNNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRR  
 20 ESVRPV

NT

**2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (nucleotide sequence)**

aagcttatggattttcaagtgcagattttcagcttctgctaatacgtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
 25 atgcagtgtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtcctgccagg  
 tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgcagcaaagt  
 aggaaggttccttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcggtggctcgggcggaggtgggtcgggt  
 ggcggcgatctcaggtgcagctgaaggagtcaggacctggcctggcggccctcacagagcctgtccatcacatgcaccgtct  
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg

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 5 gacgggcaggtcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacc  
 tcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaag  
 tgtgcagattccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcacccgcaagtcgccacgat  
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 10 ggggagacctaccagtgcagggtgacccacccccacctgccaggggccctcatgcggtccacgaccaagaccagcggcccg  
 tctgccccggaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaac  
 ttcagctgaggacatctcgggtgcagtggtgcacaacgaggtgcagctcccgacgccgggcacagcacgacgcagccccgc  
 aagaccaagggtccggcttctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccg  
 tgcagtcctgaggcagcgagccccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcgatccttcgaagctcc  
 15 catcctgggccattaccttaatctcagtaaatggaattttgtgatatgtgcctgacctactgctttgccccagatgcagagagagaag  
 gaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

AA

**2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80 (amino acid sequence)**

MDFQVQIFSFLLISASVIMSRGVDIVLTQSPASLAVSLGQRATISCRASESVEYYVTSL  
 20 MQWYQQKPGQPPKLLISAASNVESGVPARFSGSGSGTDFSLNIHPVEEDDIAMYFCQ  
 QSRKVPWTFGGGTKLEIKRGGGGSGGGSGGGGSQVQLKESGPGLVAPSQSLITC  
 TVSGFSLTGYGVNWVRQPPGKLEWLGMWGDGSTDYNSALKSRLSITKDNSKSQ  
 VFLKMNSLQTDDTARYYCARDGYSNFHYVMDYWQGTSVTVSSDHVCSRDFTP  
 PTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEG  
 25 ELASTQSELTLQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSRPS  
 PFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTS  
 TLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSR  
 DKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRA

EWEQKDEFICRAVHEAASPSQTVQRAVSVNPGKADPSKLPSWAITLISVNGIFVICCL  
TYCFAPRCRERRRRNERLRRESVRPV

NT

**500A2 scFv (nucleotide sequence)**

5 atgttgatacatctcagctccttgggcttttactcttctggatttcagcctccagaagtgcatagtgctgactcagactccagccactct  
gtctctaattcctggagaaaagagtcacaatgacctgtaagaccagtcagaatattggcacaatcttacactgggtatcaccaaaaaccaa  
aggaggctccaagggtctcatcaagtatgcttcgcagtccattcctgggatccctccagattcagtggcagtggttcggaacaga  
tttactctcagcatcaataacctggagcctgatgatacgggaatttactgtcaacaaagtagaagctggcctgtcacgttcggtcct  
ggcaccaagctggagataaaacgggggtggcggtggctcgggcggaggtgggtcgggtggcggcggtatcaggtcaagctgca  
10 gcagtcgggttctgaactagggaaacctggggcctcagtgaaactgtcctgcaagacttcaggctacatattcacagatcactatatt  
cttgggtgaaacagaagcctggagaaagcctgcagtgataggaaatgtttatgggtgaaatggtgtacaagctacaatcaaaaatt  
ccagggaaggccacactgactgtagataaaatctctagcacagcctacatggaactcagcagcctgacatctgaggattctgccat  
ctattactgtgcaagaaggccggtagcgacgggccatgctatggactactggggtcaggggatccaagttaccgtctcctctgac

AA

15 **500A2 scFv (amino acid sequence)**

MLYTSQLLGLLLFWISASRSDIVLTQTPATLSLIPGERVTMTCKTSQNIGTILHWYHQ  
KPKEAPRALIKYASQSIPGIPSRFSGSGSETDFTLSINNLEPDDIGIYYCQQSRSWPVT  
GPGTKLEIKRGGGSGGGGSGGGGSQVKLQQSGSELGKPGASVKLSCKTSGYIFTD  
HYISWVKQKPGESLQWIGNVYGGNGGTSYNQKFQGKATLTVDKISSTAYMELSSLT  
20 SEDSAIYYCARRPVATGHAMDYWGQGIQVTVSSD

NT

5' oligo:

Name : hIgAbcl5

Sequence : GTTGTTGATCAGCCAGTTCCTCAACTCCACCTACC

25 NT

3' oligo:

Name : IgA3BB

GTTGTTTTCGAAGGATCCGCGTCCACCTCCGCCATGACAACAGA

NT

5' oligo:

Name : IgGWT3

5 GTTGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

NT

3' oligo:

Name : hIgGWT5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACCTCACACATG

10 NT

5' oligo:

Name : FADD5

Sequence :

GTTGTGGATCCTTCGAACCCGTTCTGGTGCTGCTGCACTCGGTGTGCG

15 NT

3' oligo:

Name : FADD3

Sequence :

GTTGTTATCGATCTCGAGTTATCAGGACGCTTCGGAGGTAGATGCGTC

20 NT

**FADD-CSSCFV (nucleotide sequence)**

gtggatccttgaacccgttcctggtgctgctgactcgggtgctccagcctgtcagcagcgagctgaccgagctcaagttcctat  
gcctcggggcgctgggcaagcgcaagctggagcgcggtcagagcggcctagaccttctccatgctgctggagcagaacgacc  
tgagccccgggcacaccgagctcctgcgcgagctgctgcctccctgcggcgccacgacctgctcggcgcgctgcagacttcg  
25 aggcggggggcggcggccggggccgcctggggaagaagacctgtgtcagcatttaacgtcatatgtgataatgtggggaaag  
attggagaaggctggctcgtcagctcaaagtctcagacaccaagatcgacagcatcgaggacagataacccccgcaacctgacaga  
gcgtgtgcgggagtcactgagaatctggaagaacacagagaaggagaacgcaacagtggcccacctggtgggggctctcaggtc

ctgccagatgaacctgggtggctgacctggtacaagaggttcagcaggcccgtagcctccagaacaggagtggggccatgtccccg  
atgtcatggaactcagacgcatctacctccgaagcgtcctgataactcgagatcgataacaac

AA

**FADD-CSSCFV (amino acid sequence)**

5 VDPSNPFLVLLHSVSSSLSSSELTFLKFLCLGRVVGKRLERVQSGLDLFSMLLEQND  
LEPGHTELLRELLASLRRHDLRRVDDFEAGAAAGAAPGEEDLCAAFNVICDNVVGK  
DWRRLARQLKVSDTKIDSIEDRYPRNLTERVRESLRIWKNTOKENATVAHLVGALR  
SCQMNLVADLVQEVQQARDLQNRSGAMSPMSWNSDASTSEAS

NT

10 **HCD28tm5B (nucleotide sequence)**

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGTGGTTGGTGTCTGGCTTGCTATA  
GCTTG

NT

**HCD28tm3S (nucleotide sequence)**

15 GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCTATAGCA  
AGCCAG

NT

**HCD28tm5' (nucleotide sequence)**

GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT

20 NT

**HCD28tm3' (nucleotide sequence)**

GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

NT

**HCD80tm5' (nucleotide sequence)**

25

GTTGTGGATCCTCCTGCTCCCATCCTGG

NT

**HCD80tm3' (nucleotide sequence)**

GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

NT

5 **MFADD5BB (nucleotide sequence)**

GTTGTGGATCCTTCGAACCCATTCTGGTGCTGCTGCACTCGCTG

NT

**MFADD3XC (nucleotide sequence)**

GTTGTTATCGATCTCGAGTCAGGGTGTTTCTGAGGAAGACAC

10 NT

**Murine FADD nucleotide sequence** (full length, but without flanking -Ig or transmembrane sequences) (nucleotide sequence)

gtggatccttcgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctcaagt  
tcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgtcacggctgctgagcagaa  
15 cgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctggacga  
cttcgaggcggggacggcgaccgctgcgggggggggagcagatctgcaggtggcatttgacattgtgtgtgacaatgtgggg  
agagactggaaaagactggcccgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccgaagtctg  
agtgagcgggtaaggagagctgaaagtctggaagaatgctgagaagaagaacgcctcggtgccggactggtcaaggcgctg  
cggacctgcaggctgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtcccagtact  
20 aagggattcaactgtgtcttctcagaaacaccctgactcgagatcgat

AA

**Murine FADD (amino acid sequence)**

VDPSNMDPFLVLLHSLSGSLSGNDLMELKFLCRERVSKRKLERVQSGLDLFTVLLE  
QNDLERGHTGLLRELLASLRRHDLLQRLDDFEAGTATAAPPGEADLQVAFDIVCDN  
25 VGRDWKRLARELKVSEAKMDGIEEKYPRSLSERVRESLKVWKNAEKKNASVAGL  
VKALRTCRLNLVADLVEEAQESVSKSENMSPVLRDSTVSSSETP

NT

**MCASP3-5 (nucleotide sequence)**

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

NT

**MCASP3-3 (nucleotide sequence)**

5 GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

NT

**MCASP8-5 (nucleotide sequence)**

GTTGTTTCGAACATGGATTTCCAGAGTTGTCTTTATGCTATTGCTG

NT

10 **MCASP8-3 (nucleotide sequence)**

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

NT

**hcasp3-5(nucleotide sequence)**

GTTGTGGATCCTTCGAACATGGAGAACACTGAAAACCTCAGTGGAT

15 NT

**hcasp3-3 (nucleotide sequence)**

GTTGTTATCGATCTCGAGTTAGTGATAAAAATAGAGTTCTTTTGTGAG

NT

**hcasp8-5 (nucleotide sequence)**

20 GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

NT

**hcasp8-3 (nucleotide sequence)**

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTTCT

NT

25 **HuIgGMHWC (nucleotide sequence)**



gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa ctc ctg ggt  
gga ccg tca gtc ttc c

NT

**2H7-human IgE (CH2-CH3-CH4) (nucleotide sequence)**

5 aagcttgccgccatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagcttccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac  
cccccacgttcggtgctgggaccaagctggagctgaaaggtggcgggtggctcgggcgggtggatctggaggaggtgggagc  
10 tctcaggcttatctacagcagtctggggctgagctgggtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact  
tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctctgatcacgtctgctccagggaacttcacccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggca  
15 cttccccccgaccatccagctcctgtgcctcgtctctgggtacacccagggaactatcaacatcacctggctggaggacgggcaggt  
catggacgtggacttgtccaccgcctctaccacgcaggaggtgagctggcctccacacaaagcgagctcacctcagccagaag  
cactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattcc  
aaccggagaggggtgagcgcctacctaaagccggcccagccggttcgacctgttcacccgaagtcgcccacgatcacctgtctggt  
gggtggacctggcaccagcaaggggacctgaacctgacctggctccgggccaagtgggaagcctgtgaaccactccaccagaaa  
20 ggaggagaagcagcgcaatggcacgttaaccgtcacgtccacctgccggtgggcacccgagactggatcgagggggagacct  
accagtgcagggtgacccacccccacctgccaggggcctcatgcggtccacgaccaagaccagcggcccgcgtgctgccccg  
gaagtctatcggttgcgacgccggagtggccggggagccgggacaagcgcacccctcgctgcctgatccagaacttcacgtg  
aggacatctcgggtgcagtggctgcacaacgaggtgcagctcccggacgcccggcacagcacgacgcagccccgaagaccaag  
ggctccggcttctcgtcttcagccgcctggaggtgaccagggccgaatgggagcagaaaagatgagttcatctgccgtgcagtccat  
25 gaggcagcgagccccctcacagaccgtccagcgagcgggtgtctgtaaatcccgtaaatgataatctaga

AA

**2H7 scFv IgE (CH2-CH3-CH4) (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSPGKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWYIAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN

PPTFGAGTKLELKGGGSGGGGSGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
 YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
 QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSDHVCSDFTPTVKIL  
 QSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELAST  
 5 QSELTLSQKHWLSDRTYTCQVTYQGHTFEDSTKKCADSNPRGVSAYLSPSPFDLFI  
 RKSPITITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTLPVGT  
 RDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDKRTLA  
 CLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAWEQK  
 DEFICRAVHEAASPSQTVQRAVSVNPGK

10 NT

**2H7 scFv MH (SSS) MCH2WTCH3 (nucleotide sequence)**

aagcttgccgccatggattttcaagtcagatttcagcttctgctaatactgctcagtcataattgccagaggacaaattgttctctc  
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgactgcaggccagctcaagtgaagtacatgcactgg  
 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
 15 gtgggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
 tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
 ttaccagttacaatatgcactgggtaaagcagacacccatgacaggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
 20 atctgaagactctgcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacaggaccac  
 ggtcaccgtctctctgatcaggagcccaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga  
 tcgtcagttctctctccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtga  
 gccacgaagaccctgaggtcaagttcaactggtagtggtgacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggctc  
 25 tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtgga  
 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggactccgacggctccttctctctacagca  
 agctcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcagaggtctgcacaaccactacacg  
 cagaagagcctctccctgtctccgggtaaatgatctaga

AA

**2H7 scFv MH (SSS) MCH2WTCH3 (amino acid sequence)**

MDFQVQIFSLLISASVIIARGQIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQ  
QKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFN  
5 PPTFGAGTKLELKDGGGSGGGGSGGGGSSQAYLQQSGAELVRPGASVKMSCKASG  
YTFTSYNMHWVKQTPRQGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYM  
QLSSLTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSSDQEPKSSDKTHTSP  
SPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV  
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA  
10 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP  
VLDSGDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

NT

**5B9 scFv MTHWTCH2CH3 (nucleotide sequence)**

aagcttgccgcatgaggttctctgctcagcttctgggctgcttgctctggatccctggatccactgcagatattgatgacgcag  
15 gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta  
tttgattggtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtcccagacaggttc  
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaatc  
agaacttccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcggtggctcggcggtgggtgggtcgggtg  
cggcggtatcgtcacaggtgcagctgaagcagtcaggacctggcctagtcagtcctcacagagcctgtccatcacctgcacagtct  
20 ctggtttctcattaactacctatgctgtacactgggttcgccagctcctcaggaaagggtctggagtggctgggagtgatatggagtgt  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt  
ctgcaacctaatgacacagccatttattactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggtcaa  
ggaacctcagtcaccgtctcctctgatcaggagcccaaatcttgacaaaactcacacatccccaccgtccccagcacctgaactcc  
tggggggaccgtcagcttctccttctcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtg  
25 tggacgtgagccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
ggaggagcagtaaacagcacgtacctgtgtgtcagcgtcctaccgtcctgcaccaggactgggtgaatggcaaggagtacaag  
tgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgt  
acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc

tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

AA

**5B9 scFv MTHWTCH2CH3 (amino acid sequence)**

5 MRFSAQLLGLLVLWIPGSTADIVMTQAAFSNPVTLGTSASISCRSSKSLHNSNGITYL  
YWYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCA  
QNLELPLTFGAGTKLELKRGGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSIT  
CTVSGFSLTTYAVHWVRQSPGKGLEWLGVIWSGGITDYNAAFISRLSITKDDSKSQV  
FFKMNSLQPNDAIYYCARNGGDNYPYYYAMDYWGQGTSTVTVSSDQEPKSSDKT  
10 HTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD  
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT  
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

15 **1. 2H7 scFv with alternative VHL11 mutations:**

Nucleotide sequence

Aagcttgccgccatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgcagggccagctcaagtgttaagttacatgactgg  
taccagcagaagccaggatctccccaaacctggatttatgcccataccacctggcttctggagtcctgctgcttcagtgga  
20 gtgggtctgggacctctactctctcacatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggtgag (one of the following: ten, acn, gan, can, aan, cgn, agn)  
gtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacatttaccagttacaatatgactgggtaaacgagacac  
ctagacagggcctggaatggattggagctatttatccaggaaatggtgatacttctacaatcagaagttcaagggaaggccacact  
25 gactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcgggtctatttctgtgcaagagt  
gtgtactatagtaactcttactgttacttcgatgtctggggcacagggaccacgggtcaccgtctcttctgatcag

Amino acid sequence

mdfqvqifsflisaviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparfsg  
30 ssgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgae (one of the  
following: s, t, d, e, q, n, r, k, h)  
vrpgasvkmsckasgytftsynmhwwkqtrpglewigaiypngdtsynqkfkkgkatltvdkssstaymqllslltsedsav  
fcarvvyysnsywyfdvwgtgtvtvssdq

35 **2. VHL11 deletion**

Nucleotide sequence:

Aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
5 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgaggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatta  
ccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatacttc  
ctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacat  
10 ctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccacgg  
tcaccgtctcttctgatcag

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparsfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtklekdggsgggsgggssqaylqqsgaevrpgasvkmsckas  
15 gyftsynmhwwkqtrpqlgwigaiypngdtsynqkfkglatltvdksstaymqlssltedsavyfcarvvyysnsywy  
fdvwgtgtvtvssdq

### 3. 2H7 VL L106 with alternative mutations

Nucleotide sequence:

20 aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
25 ccaccacgttcggtgctgggaccaagctggag (tcn, agn, aan, cgn, can, gan, and non-natural  
derivatives of these codons) aaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagctc

Amino acid sequence:

Mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparsfsg  
gsgsgtsysltisrveadaatyycqqsfnpptfgagtkle (s, t, r, k, h, q, n, d, e, and non-natural  
30 derivatives of these amino acids at position 106)kdggsgggsgggss

### 4. VL L106 deletion

Nucleotide sequence:

35 Aagcttgccgcatggattttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
40 ccaccacgttcggtgctgggaccaagctggagaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagctc

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiapsnlasgvparsfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtklekdggsgggsgggss

### 45 5. IgE CH3 CH4

Nucleotide sequence:

tccaacccgagaggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtct  
 ggtggtggacctggcaccagcaaggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccag  
 aaaggaggagaagcagcgcgaatggcacgttaacctgacgtccaccctgccggtgggcacccgagactggatcgagggggaga  
 cctaccagtgcagggtgacccacccccacctgcccaggccctcatgcggtccacgaccaagaccagcgcccgcgtgtctccc  
 5 cggaaagtctatgcgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcc  
 tgaggacatctcggtgcagtggtgcacaacgaggtgcagctcccggacggccggcacagcacgacgcagccccgcaagacca  
 agggctccggcttcttcttcttcagccgcctggaggtgaccaggccgaatgggagcagaaagatgagttcatctgccgtgcagtc  
 catgaggcagcgagccccctacagaccgtccagcgagcgggtgtctgtaaatcccgttaataatgataatctagaa

10 Amino acid sequence:  
 snprgvsaylsrpspfdlfrksptitclvdlapskgtvnltwsrasgkpvnhstrikeekqrmgtltvtstlpvgtrdwiegetyqcr  
 vthphlpralmrstktsgpraapevyafatpewpgsrdrklaciqnfmpedisvqwlhnevqlpdarhsttqprktksgffv  
 fsrlevtraeweqkdeficravheaaspsqtvqravsvnpgk

## 15 6. hIgG1H/IgE WCH3 WCH4

Nucleotide sequence:

tgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta  
 gccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacctggcaccagcaaggggac  
 cgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgcgaatggcacgtt  
 20 aaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtgacccacccccacct  
 gccagggccctcatgcggtccacgaccaagaccagcgcccgctgtctgccccggaagtctatgcgtttgcgacgccggagt  
 gccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctcggtgcagtggtgcacaac  
 gaggtgcagctcccgacggccggcacagcacgacgcagccccgcaagaccaagggtccggcttcttcttcttcagccgcctg  
 gaggtgaccaggggccgaatgggagcagaaagatgagttcatctccgtgcagtcctatgaggcagcgagccccctacagaccgtc  
 25 cagcgagcgggtgtctgtaaatcccgttaataatgataatctagaa

Amino acid sequence:

dqepkssdkthtspspasnpgrvsaylsrpspfdlfrksptitclvdlapskgtvnltwsrasgkpvnhstrikeekqrmgtltvt  
 stlpvgtrdwiegetyqcrvthphlpralmrstktsgpraapevyafatpewpgsrdrklaciqnfmpedisvqwlhnevqlp  
 30 darhsttqprktksgffvfsrlevtraeweqkdeficravheaaspsqtvqravsvnpgk

## 7. IgE WCH2 WCH3 WCH4

Nucleotide sequence:

Tgatcacgtctgtccagggaacttccccgcccaccgtgaagatcttacagtcgtcctgcgacggcggcgggcacttccccccga  
 35 ccatccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtg  
 gacttgtccaccgcctctaccacgcaggaggggtgagctggcctccacacaaagcgagctcacctcagccagaagcactggctgt  
 cagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccagaagagtgatgcagattccaacccgaga  
 ggggtgagcgcctacctaagccggcccagcccgttcgacctgttcatccgcaagtcgcccacgatcacctgtctggtggtggacct  
 ggcaccagcaagggggaccgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggaga  
 40 agcagcgcgaatggcacgttaacctgacgtccaccctgccggtgggcacccgagactggatcgagggggagacctaccagtga  
 gggtgacccacccccacctgcccaggccctcatgcggtccacgaccaagaccagcgcccgctgtctgccccggaagtctatg  
 cgtttgcgacgccggagtggccggggagccgggacaagcgcaccctcgcctgcctgatccagaacttcatgcctgaggacatctc  
 ggtgcagtggtgcacaacgaggtgcagctcccggacggccggcacagcacgacgcagccccgcaagaccaagggtccggc  
 ttcttcttcttcagccgcctggaggtgaccaggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctatgaggcagc  
 45 gagccccctacagaccgtccagcgagcgggtgtctgtaaatcccgttaataatgataatctaga

Amino acid sequence:

dhvcsrdftptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmdivlstaattqegelastqseltlsqkhwlslrt  
yteqvtyqghtfedstkkcadsnprgvsaylsrpspfdlfrksptitclvdlapskgtnltwsrasgkpvnhstrkeekqrmgtl  
5 tvstlpvgtrdwiegetyqcrvthphlpralmrsttktsgraaapevyafatpewpgsrdrklaciqnfmpeidisvqwlhnev  
qlpdarhsttqprktkgsffvfrlevtraeweekdeficravheaaspsqtvqravsvnpgk

#### 8. hIgG1H/IgE CH3 CH4 (ORF)

Nucleotide sequence:

Nucleotide sequence:

10 tgatcaggagcccaaatctctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggtgagcgcctaccta  
gccggccagcccggttcgacctgttcacccgcaagtcgcccacgatcacctgtctggtggtggacctggcaccagcaaggggac  
cgtgaacctgacctggtcccgggccagtggaagcctgtgaacctccaccagaaaggaggagaagcagcgaatggcacgtt  
aaccgtcacgtccacctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtgacccacccccac  
15 gccaggggccctcatcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatcggttgcgacgccggagt  
gccggggagccgggacaagcgcacccctgcctgcctgatccagaacttcacgtgaggacatctcggtgcagtggctgcacaac  
gaggtgcagctcccgagcggcgacacgacgacgagccccgcaagaccaagggtccggcttctcgtctcagccgcctg  
gaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctgaggcagcagccctcacagaccgtc  
cagcgagcgggtgtctgtaaatcccggtaaagcggatcctcgaa

20 Amino acid sequence:

dqepkssdkthtspspasprgvsaylsrpspfdlfrksptitclvdlapskgtnltwsrasgkpvnhstrkeekqrmgtlvt  
stlpvgtrdwiegetyqcrvthphlpralmrsttktsgraaapevyafatpewpgsrdrklaciqnfmpeidisvqwlhnevqlp  
darhsttqprktkgsffvfrlevtraeweekdeficravheaaspsqtvqravsvnpgksgsfe

#### 25 9. 2H7 VHL11S scFv hIgG1(SSS-S)H hIgE WCH3 WCH4

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctc  
ccagctccagcaatctgtctgcacatccaggggagaagggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtggca  
30 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggtgatact  
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
35 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctctctgatcaggagcccaatctctgacaaaactcacacatccccaccgtcctcagcatccaacccgagaggggt  
gagcgcctacctaagccggccagcccggttcgacctgttcacccgcaagtcgcccacgatcacctgtctggtggtggacctggcac  
ccagcaaggggaccgtgaacctgacctgggtcccgggccagtggaagcctgtgaacctccaccagaaaggaggagaagcag  
cgcaatggcacgttaacctgcacgtccacctgccggtgggcacccgagactggatcgagggggagacctaccagtgcagggtg  
40 accacccccacctgccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgttg  
cgacggcgagtgccggggagccgggacaagcgcacccctgcctgcctgatccagaacttcacgtgaggacatctcggtgc  
agtggctgcacaacgaggtgcagctcccgagcggcgacacgacgacgagccccgcaagaccaagggtccggcttctc  
gtcttcagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctgaggcagcagcc  
cctcacagaccgtccagcgagcgggtgtctgtaaatcccggtaaatgataatctaga

45

Amino acid sequence:

Mdfqvqifsflisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfs  
 gsgsgtsysltisrveaadaatyycqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsck  
 asgytftsynmhwwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyysnsy  
 wyfdvwtgtttvtvssdqepkssdkthtsppssasnprgvsaylsrpspfdlfrksptitclvvdlapskgtnlwsrasgkpv  
 5 nhstrkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktsgpaaapevyafatpewpgsrdrkrtlacliqnfm  
 pedisvqwlhnevqlpdarhsttqprktksgffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgk

# 10. 2H7 VHL11S scFv hIgG1(SSS-P)H hIgE WCH3 WCH4

## 10 Nucleotide sequence:

aagcttgccgcatggaatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
 ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggtatctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
 15 ccacccacgttcgggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
 tctcaggcttatctacagcagctctggggctgagtcggtagggcctggggcctcagtgaaagatgctcgtcaaggcttctggctacacat  
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatgattggagctatttatccaggaaatggtgatact  
 tctacaatcagaagttaagggaagccacactgactgtagacaatctccagcagacgctacatgcagctcagcagcctgac  
 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactggtacttcgatgtctggggcacaggggaccac  
 20 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcatccaacccgagaggggt  
 gagcgctacctaagcggccagccgcttcgacctgttcacgcaagtcgcccacgatcacctgtctggtggtggacctggcac  
 ccagcaaggggaccgtgaacctgacctgggtcccgggccagtgagggaagcctgtgaaccactccaccagaaaggaggagaagcag  
 cgcaatggcacgttaaccgtcacgtccaccctgccggtgggacccgagactggatcgagggggagacctaccagtgagggtg  
 accacccccacctgcccagggccctcatgcggtccacgaccaagaccagcggccgcgtgctgccccggaagtctatgcgtttg  
 25 cgacgccggagtggccggggagccgggacaagcgcacctcgcctgcctgatccagaacttcagctgaggacatctcgggtgc  
 agtgggtgcacaacgaggtgcagctcccgagcggcgacagcagcagcagccccgcaagaccaaggggtccgggtctctc  
 gtcttcagccgctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctatgaggcagcagcc  
 cctcacagaccgtccagcagcgggtgtctgtaaatcccggtgaatgataatctaga

## 30 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
 ssgsgtsysltisrveaadaatyycqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
 gytftsynmhwwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyysnsywy  
 fdvwtgtttvtvssdqepkssdkthtsppspasnprgvsaylsrpspfdlfrksptitclvvdlapskgtnlwsrasgkpv  
 35 trkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktsgpaaapevyafatpewpgsrdrkrtlacliqnfmpe  
 disvqwlhnevqlpdarhsttqprktksgffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgk

# 10. 2H7 VL L106S

aagcttgccgcatggaatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
 40 ccagctctccagcaatcctgtctgcattctccaggggagaaggtcacaaatgacttgaggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggtatctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
 gtgggtctgggacctcttactcttcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
 ccacccacgttcgggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
 tc

45



Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleskdggsgggsgggss

5     **11. 2H7 VL L106S scFv**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaagggtcaccaatgacttgcagggccagctcaagttaagttacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
10     gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtgctgggaccaagctggagttctaaagatggcggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
15     atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcag

Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
20     sgsgtsysltisrveadaatyycqqsfnpptfgagtkleskdggsgggsgggssqaylqqsgaelvrpgasvkmscka  
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy  
fdvwgtgtvtvssdq

25     **12. 2H7 scFv VL L106S VHL11S scFv**

Nucleotide sequence:

Aagcttgccgccatggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaagggtcaccaatgacttgcagggccagctcaagttaagttacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
30     gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtgctgggaccaagctggagttctaaagatggcggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctgggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
35     ggtcaccgtctcttctgatcag

Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleskdggsgggsgggssqaylqqsgaesvrpgasvkmscka  
40     sgytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy  
yfdvwgtgtvtvssdq

45     **10. Human IgD hinge linker with attached restriction sites**

Nucleotide:

gtggatccaggttcgaagtctccaaaggcacaggcctcctccgtgccactgcacaaccccaagcagagggcagcctcgccaagg  
caaccacagccccagccaccaccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaagaggaacaag  
aagagagagagacaaagaccggtgcagtcgacg

- 5 Amino acid:  
vdpgskspkaqassvptaqpqaegslakattapatttrntgrggeekkkkekeeeqeeretktgavd

**Sequence of Native IgD hinge domain:**

(includes a cysteine residue—we truncated the hinge prior to that residue for these  
10 constructs:)

Nucleotide:

gagtctccaaaggcacaggcctcctccgtgccactgcacaaccccaagcagagggcagcctcgccaaggcaaccacagcccca  
gccaccaccgtaacacaggaagaggaggagaagagaagaagaaggagaaggagaaagaggaacaagaagagagagagac  
aaagacaccagagtgtccgagccacaccagcctcttggcgtctacctgtaaccct

- 15 Amino acid sequence:  
Espkaqassvptaqpqaegslakattapatttrntgrggeekkkkekeeeqeeretktpecpshtqplgvyltp

**12. 2H7 VH L11S**

- 20 Nucleotide sequence:

caggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacattta  
ccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggatgatacttc  
ctacaatcagaagtcaaggggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacat  
ctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccacgg  
25 tcaccgtctcttct

Amino acid sequence:

qaylqqsgaesvrpgasvkmckasgytftslnmhvkwqtpqrqglewigaiypngdtsynqkfkkgkatltvdkssstaym  
qlssltsedsavyfcarvvyysnsywyfdvwtgtvtvss

- 30

**13. 2H7 VH L11S scFv**

Nucleotide sequence:

- aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcactctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg  
35 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgaggttttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggatgatact  
40 tctacaatcagaagtcaaggggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcag

Amino acid sequence:

- 45 mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqwsfnpptfgagtklelkdggsggggsggggssqaylqqsgaesvrpgasvkmckas

gytftsynmhvwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyyysnsywy  
fdvwgtgtvtvssdq

5 **14. 2H7 scFv VH L11S hIgG1 (CSC-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtggca  
10 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacggttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggtatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctctgtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatt  
taccagttacaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatactt  
cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgaca  
15 tctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccacg  
gtcaccgtctctctgtatcaggagcccaatcttgacaaaactcacacatctccaccgtgctcagcacctgaactcctgggtggacc  
gtcagttctctcttccccccaaaacccaaggacacctcatgatctccggaccttgaggtcacatgcgtggtggtggacgtgag  
ccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca  
gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtct  
20 ccaacaagccctccagccccatcgagaaaacaatctcaaagccaaaggcgagccccgagaaccacaggtgtacacctgc  
ccccatcccggtgagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccaagcgacatcgccgtggag  
tgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa  
gtcaccgtggacaagagcaggtggcagcaggggaacgtctctcatgctccgtgatgcaggtctgcacaaccactacacgc  
agaagagcctctccctgtctccggtaaatgatctaga

25 Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhvwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyyysnsywy  
30 fdvwgtgtvtvssdqepkscdkthtsppcsapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltc  
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

**15. 2H7 scFv VH L11S IgE WCH2 WCH3 WCH4**

35 Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctcccccaccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtggca  
40 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacggttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggtatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctctgtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatt  
taccagttacaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatactt  
cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgaca  
tctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccacg  
45 gtcaccgtctctctgtatcacgtctgtccagggacttcacccgccaccgtgaagatcttacagtcgtcctgcacggcgggcggg  
acttccccccgaccatccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcagg

tcatggacgtggacttgtccaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcacctcagccagaa  
 gcaactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattc  
 caaccgagaggggtgagcgccctacctaagccggccagccggttcgacctgttcacccgaagtcgccacgatcacctgtctgg  
 5 tgggtggacctggcaccagcaaggggaccgtgaacctgacctggctccggggccagtgggaagcctgtgaacctccaccagaa  
 aggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagacc  
 taccagtgcaggggtgacccaccccccacctgccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccg  
 gaagtctatgcgttgcgacgccggagtggccggggagccgggacaagcgacccctcgctgcctgatccagaactcatgcctg  
 aggacatctcggtgcagtggctgcacaacgaggtgcagctccggagcggcgacagcacgacgcagccccgaagaccaag  
 ggctccggcttctcgtcttcagccgctggaggtgaccagggccgaatgggagcagaaaagatgagttcatctgccgtgcagtcct  
 10 gaggcagcgagccctcacagaccgtccagcgagcggtgtctgtaaaccggtaaatgataatctaga

Amino acid sequence:

mdfvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
 ssgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
 15 gyftsynmhwwkqtrprglewigaiypngdtsynqfkkgkatltvdksstaymqlssltedsavvyfcarvvyysnsywy  
 fdvwtgtttvtssdhvcsrdftptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmdivlstatteqegelastq  
 seltslqkhwlsdrtytcvtyqghtfedstkkcadsnprgvsaylsrpsfdlfrksptitclvdlapskgtvnlwsrasgkpv  
 nhstrkeekqrmgtltvtstlpvgrdwiegetyqcrvthphlpralmrsttktspraapevyafatpewpgsrdrklaciqnfm  
 pedisvqwlhnevqlpdarhsttqprktksgffvrsrlevtraeweqkdeficravheaaspsqtvravsvnpgk  
 20

## 16. 2H7 scFv VH L11S mIgE WCH2 WCH3 WCH4

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgtaatacgtgcttcagtcataattgccagaggacaaattgttctctc  
 ccagctcagcaatcctgtctgcattctccaggggagaagggtcacaatgacttcgaggccagctcaagtgtaaattacatgcactgg  
 25 taccagcagaagccagatcctccccaaacctggatttatgccccatccaacctggcttctggagtcacctgctcgttcagtggca  
 gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggagtttaac  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
 tctcaggttatctacagcagctctggggctgagctgtgaggcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatt  
 taccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatactt  
 30 cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagtcagcagcctgaca  
 tctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggacttcgatgtctggggcacagggaccacg  
 gtcaccgtctctctgacacgttcgacctgtcaacatcactgagcccaccttgaggctactccattcatcctgcgaccccaatgcattcc  
 actccaccatccagctgtactgcttcatttatggccacatcctaaatgatgtctctgtcagctggctaattggacgatcgggagataactg  
 atacacttgcaaaactgttctaataaggaggaaggcaaaactagcctctacctgcagtaaaactcaacatcactgagcagcaatggat  
 35 gtctgaaagcaccttcacctgcaaggtcacctcccaaggcgtagactatttgccacactcgagatgccagatcatgagccacg  
 gggtgtgattacctacctgatccaccagccccctggacctgtatcaaaacgggtgctcccaagcttacctgtctggtggtggacctg  
 gaaagcgagaagaatgtcaatgtgacgtggaaccaagagaagaagacttcagctcagcatccagtggtactaagcaccacaa  
 taagccacaactagtatcacctccatcctgctgtagtggcaaggactggattgaaggctacggctatcagtgcatagtggaccac  
 cctgattttcccaagccattgtgcgtccatcaccaagacccagggcagcgctcagccccgaggtatatgtgtccaccaccag  
 40 aggaggagagcgaggacaaacgcacactcacctgtttgatccagaacttcttccctgaggatatctctgtgcagtggctgggggatg  
 gcaaaactgatcacaacagccagcacagtaccacaacacctgaaatccaatggctccaatcaaggcttcttcatcttcagtcgcta  
 gaggtcgcaagacactctggacacagagaaaacagttcacctgccaaagtatccatgaggcacttcagaaaccaggaaactgg  
 agaaaaacaataccacaagccttgtaaacctccctccgtccatcctagtaatatagag

45 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytfts ynmhvwkqtrprglewigaiypngdtsynqkfk gkatltvdkssstaymqlssltedsavyfcarvvyyysnsywy  
fdvwtgtttvtvssdhvrpvniteptlellhsscdpnafhstiqlycfiyghilndvsvswlmddreitdlaqtvlkeegklastcs  
5 klniteqqwmsestftckvtsqgvdyahtrrcpdheprgvitylippspldlyqngapklclvvdleseknvnvtwnqekkts  
vsasqwyt khnnattsitsilpvvakdwiegygyqci vdhpdfpkpivrsitktpgqrsapevyvfpppeesedkrtltcliqn  
ffpedisvqwlgdgklisnsqhssttp lksnsgnqgffisrlevaktlwtqrkqftcqihealqkprklektistslgntslrps

### 17. 2H7 scFv VH L11S hIgA WH WCH2 T4CH3

10 Nucleotide sequence:

aagcttgccgcatggaatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatctctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
15 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctctgtaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatt  
taccagttacaatatgcactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatactt  
cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgaca  
tctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttacttggtacttcgatgtctggggcacagggaaccag  
20 gtcaccgtctctctgtatcagccagttccctcaactccactacccatctccctcaactccactacccatctccctcatgctgccacc  
cccactgtcactgcaccgaccggcctcagaggactgctcttaggttcagaagcgaatcctcacgtgcacactgaccggcctgaga  
gatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgtgttcaaggaccacctgaccgtgacctctgtggctgc  
tacagcgtgtccagtgctcctgccgggctgtgccgagccatggaaccatgggaagaccttacttgactgctgcctaccccgagtc  
aagaccccgtaccgccaccctctcaaaatccggaacacattccggccgaggtccacctgctgccgccgctcggaggagc  
25 tggcctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggctgcaggggtca  
caggagctgccccgcgagaagtacgtgacttgggcatccggcgaggagccagccagggcaccaccaccttcgctgtgaccagc  
atactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggggccacgaggcctgccgtggccttca  
cacagaagaccatcgaccgcttggcgggtaaacccacccatgtcaatgtgtctgttgcagtgagggtggactgataatctaga

30 Amino acid sequence:

mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytfts ynmhvwkqtrprglewigaiypngdtsynqkfk gkatltvdkssstaymqlssltedsavyfcarvvyyysnsywy  
fdvwtgtttvtvssdqpvpstptpspstpccchprlsrlhpaledlllgseailtctltglrdasgvtftwtpssgksavqgp  
35 pdrldcgcysvssvlp gcaepwnhgtftctaaypesktpltatlsksgntfrpevhllpppseelalnelvtltclargfspkdvivr  
wlqgsqelprekyltwasrqepsqgtttfavtsilrvaedwkkgdtfscmv ghealplafqtidrlagkpthvvnsvvmaev  
d

### 18. 2H7 scFv VH L11S mIgA WH WCH2 T4 CH3

40 Nucleotide sequence:

aagcttgccgcatggaatttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatctgtctgcattctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatctctccccaaccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
45 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctctgtaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacatt

taccagttacaatatgactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatactt  
 cctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgaca  
 tctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccacg  
 gtcaccgtctcttctgatcacatctgttctcctactactcctcctccaccttctgccagcccagcctgtcactgcagcggccagctc  
 5 ttgaggacctgctcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgagggagctgtcttcacctggga  
 gccctccactgggaaggatgcagtgcagaagaaagctgtgcagaattcctgcggctgctacagtgtgtccagcgtcctgcctggctg  
 tctgagcgtggaacagtggcgcacattcaagtgcacagttaccatcctgagctgtacaccttaactggcacaattgccaaagtc  
 acagtgaacaccttccccacccaggtccacctgctaccgccgccgtcggaggagctggccctgaatgagctcgtgtccctgacatg  
 cctggtgcgagctttcaaccctaaagaagtgtgtgcgatggctgcatggaaatgaggagctgtcccagaaagctacctagtgtt  
 10 gagccctaaaggagccagggcagggagccaccacctacctggtgacaagcgtgttgcgtgtatcagctgaaatctggaacagg  
 gtgaccagtactcctgcatggtgggccacgaggccttggccatgaacttaccacagaagaccatcgaccgtctgtcgggtaaaccaca  
 ccaatgtcagcgtgtctgtgatcatgtcagaggagattgataatctagat

Amino acid sequence:

15 mdfqvqifsflisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwi yapsnlasgvparfsg  
 sgsqtsysltisrveadaatyccqwsfnpptfagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
 gytfitsymhwwkqtrpqglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltedsavyfcarvvyysnsywy  
 fdvwtgtttvssdhicspptppppscqpslsqrpaledlllgdsasitctlnlrdpegavftwepstgkdavqkkavqnsccg  
 cysvssvlpgcaerwnsgasfkctvthpesdltltgiakvtvntfppqvhlpppseelalnelvslclvrafnpkevlvrwlhgn  
 20 eelspesylvfeplkepegattylvtsvlrvsaeiwwkqgdqyscmvghealpmnftqktidrlsgkptnsvsvimsegd

#### A. mIgA WCH2 T4CH3

Nucleotide sequence:

Gttgttgatcacatctgttctcctactactcctcctccaccttctgccagcccagcctgtcactgcagcggccagctcttgaggac  
 25 ctgctcctgggttcagatgccagcatcacatgtactctgaatggcctgagagatcctgagggagctgtcttcacctgggagccctcca  
 ctgggaaggatgcagtgcagaagaaagctgtgcagaattcctgcggctgctacagtgtgtccagcgtcctgcctggctgtgctgagc  
 gctggaacagtggcgcacattcaagtgcacagttaccatcctgagctgtacaccttaactggcacaattgccaaagtcacagtga  
 caccttccccacccaggtccacctgctaccgccgccgtcggaggagctggccctgaatgagctcgtgtccctgacatgcctggtgc  
 gagctttcaaccctaaagaagtgtgtgtgcgatggctgcatggaaatgaggagctgtcccagaaagctacctagtgtttgagccct  
 30 aaaggagccagggcagggagccaccacctacctggtgacaagcgtgttgcgtgtatcagctgaaatctggaacaggggtgaccag  
 tactcctgcatggtgggccacgaggccttggccatgaacttaccacagaagaccatcgaccgtctgtcgggtaaaccaccaatgtc  
 agcgtgtctgtgatcatgtcagaggagattgataatctagat

Amino acid sequence:

35 dhicspptppppscqpslsqrpaledlllgdsasitctlnlrdpegavftwepstgkdavqkkavqnsccgysvssvlpgcaer  
 wns gasfkctvthpesdltltgiakvtvntfppqvhlpppseelalnelvslclvrafnpkevlvrwlhgneelspesylvfeplk  
 epegattylvtsvlrvsaeiwwkqgdqyscmvghealpmnftqktidrlsgkptnsvsvimsegd

#### 20. K322S CH2 region

Nucleotide sequence:

40 cctgaactcctggggggaccgtcagttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacat  
 gcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttaactggtagctggacggcgtggaggtgcataatgccaagac  
 aaagccgcgggaggagcagtacaacagcacgtaccgtgtgtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa  
 ggagtacaagtgtcgtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaa

45 Amino acid sequence:



ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagttaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggaccac  
 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
 5 ccgtcagttcttcttccccccaaaacccaaggacacccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtga  
 gccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgtcggctt  
 ccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc  
 ccccatccccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag  
 10 tgggagagcaatgggcagccgggagaaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa  
 gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgc  
 agaagagcctctccctgtctccgggtaaatgatctaga

**Amino acid sequence:**

15 mdffvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwi yapsnlasgvparfsg  
 sgsdgsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
 gyftsynmhvkwqtprrglewigaiypngdtsynqkfkgkatltvdksstaymqlssltedsavyfcarvvysnsywy  
 fdvwtggtvtvssdqepkssdkthtsppssapellggpsvflfppkpdltlmisrtpvctvvvdvshedpevkfnwyvdgv  
 evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykcsvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsltc  
 20 lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

**23. 2H7 scFv VHL11S hIgG1 (SSS-S)H K322LCH2 WCH3**

**Nucleotide sequence:**

25 aagcttgccgcatggtatttcaagtgcagatttcaagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggtacctccccaaaccctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga  
 gtgggtctgggaccttactcttcacaaatcagcagagtggaggtgaagatgctgccacttattactgccagcagtgagggtttaa  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
 30 tctcaggcttatctacagcagctctggggtgagtcggtgaggcctggggcctcagtgagatgtcctgcaaggcttctggctacacat  
 ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
 tcctacaatcagaagttaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggaccac  
 ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
 35 ccgtcagttcttcttccccccaaaacccaaggacacccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtga  
 gccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcctggtct  
 ccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc  
 ccccatccccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag  
 40 tgggagagcaatgggcagccgggagaaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa  
 gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgc  
 agaagagcctctccctgtctccgggtaaatgatctaga

**Amino acid sequence:**

45 mdffvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwi yapsnlasgvparfsg  
 sgsdgsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas



gytftsynmhvwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltsedsavyfcarvvyysnsywy  
fdvwtgttvtvssdqepkssdkthtsppssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykclvsnkalspapiektiskakgqprepvytlppsrdeltknqvsltcl  
vkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslspsgk

5

#### 24. 2H7 scFv VHL11S hIgG1 (CSS-S)H K322SCH2 WCH3

Nucleotide sequence:

aagcttgccgcatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttcagggccagctcaagtgaagtacatgcactgg  
10 taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggaccttactctctcacatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggttatctacagcagctctggggctgagtcggtgagcgctggggcctcagtgaaagatgctcgaaggcttctgctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcgctggaatggattggagctatttatccaggaaatggtgatact  
15 tctacaatcagaagtcaagggaagggcacactgactgtagacaaaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
ccgtcagttctcttctccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcggtggaggtgcataatgccaagacaaagccgaggaggagc  
20 agtacaacagcagctaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgtcgtgct  
ccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc  
ccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatcccagcgacatcgccgtggag  
tgaggagcaatgggcagccggagaacaactacaagaccacgcctccgctgctggactccgacggctccttctctctacagcaa  
gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgc  
25 agaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
30 gytftsynmhvwkqtrpqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltsedsavyfcarvvyysnsywy  
fdvwtgttvtvssdqepkssdkthtsppssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykcsvsnkalspapiektiskakgqprepvytlppsrdeltknqvsltc  
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslspsgk

#### 35 25. P331S CH2

Nucleotide sequence:

cctgaactcctggggggaccgtcagttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacat  
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcggtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtagaacagcagcagctaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa  
40 ggagtacaagtgaaggtctccaacaagccctccagcctccatcgagaaaacaatctccaaagccaaa

Amino acid sequence

Pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlng  
45 keykckvsnkalspasiectiskak

**26. P331S CH2 WCH3**

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacat  
gcgtgggtggacgtgagccacaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac  
5 aaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaa  
ggagtacaagtcaaggtctccaacaaagccctcccagcctccatcgagaaaacaatctccaaagccaaagggcagccccgaga  
accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatc  
ccagcgacatcgccgtggagtgaggagcaatgggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgac  
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag  
10 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

15 Amino acid sequence

pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw  
ngkeykckvsnkalpasiektiskakgqprepvytlppsrdektknqvsitclvkgfypsdiavewesngqpennykttppvl  
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

**20 27. 2H7 scFv VH L11S (SSS-S)H P331S CH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctctgcttcagtgga  
25 gtgggtctgggaccttctactcttcacaatcagcagagtgaggagctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctctgaaagcttctggctacacat  
ttaccagttacaatatgactgggtaaagcagacacctagacaggcctggatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagtcaagggaagccacactgactgtagacaatctccagcacagcctacatgcagctcagcagcctgac  
30 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactgttacttcgatgtctggggcacaggggaccac  
ggtcaccgtctctctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
ccgtcagttctcttctcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc  
35 tccaacaaagccctcccagcctccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgc  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtggag  
tgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttctctctacagcaa  
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgc  
agaagagcctctccctgtctccgggtaaatgatctaga

40

Amino acid sequence

mdfvqvqifsfllisasviiargqivlsqspailsaspgekvtmtrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrpqglewigaiypngdtsynqkfkgtatltvdkssstaymqlssltedsavyfcarvvyysnsywy  
45 fdvwtgtttvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv

evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpasiektiskakgpprepqvylppsrdeitknqvsltc  
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhlealnhhtqkslsispkg

5     **28.     2H7 scFv VH L11S (CSS-S)H P331S CH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagtgttaagtacatgcactgg  
taccagcagaagccaggaatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
10     gtgggtctgggaccttctactctcacaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacggttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagtcaagggaagccacactgactgtagacaatctccagcagacgctacatgcagctcagcagcctgac  
15     atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaaccac  
ggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
ccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga  
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggagagc  
agtacaacagcagctaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc  
20     tccaacaaagccctcccagcctccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctgc  
ccccatcccggtgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcagacatcgccgtggag  
tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaa  
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcaggtctgcacaaccactacacgc  
agaagagcctctccctgtctccgggtaaatgatctaga

25

Amino acid sequence

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdksstaymqlssltedsavfyfcarvvyysnsywy  
30     fdvwtgtttvtssdqepkscdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpasiektiskakgpprepqvylppsrdeitknqvsltc  
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhlealnhhtqkslsispkg

35     **29.     T256N CH2 region**

Nucleotide sequence:

Cctgaactcctggggggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggaacctgaggtcaca  
tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaga  
caaagccgaggagagcagtaacagcagctaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaa  
ggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaa

40

Amino acid sequence

pellggpsvflfppkpkdtlmisrnpvtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwl  
lngkeykckvsnkalpapietiskak

45     **30.     T256N CH2 WCH3**

Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttcccccaaaaccaaggacacctcatgatctcccgaacctgaggtcacat  
gcgtgggtggtggacgtgagccacgaagacctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaa  
ggagtacaagtgaaggttccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgaga  
5 accacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatc  
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac  
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctctcatgctccgtgatgcatgag  
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

10

#### Amino acid sequence

pellggpsvflfppkpkdtlmisrnpvctcvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw  
lngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitclvkgfypsdiavewesngqpennyktpvp  
15 ldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispk

### 31. 2H7 scFv VH L11S (SSS-S)H T256N CH2 WCH3

#### Nucleotide sequence:

20 aagcttgccgccatggattttcaagtcagatttctcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgaagtacatgcactgg  
taccagcagaagccaggatcctccccaaacctggatttatccccatccaacctggcttctggagtcctgctgcttcagtgga  
gtgggtctgggacctctactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaagttaac  
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
25 tctcaggcttatctacagcagctctggggtgagtcggtagggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggatggattggagctatttatccaggaaatgggtgatact  
tctacaatcagaagtcaagggaagggcacactgactgtagacaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctctctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
30 ccgtcagttctcttcccccaaaaccaaggacacctcatgatctcccgaacctgaggtcacatgcgtgggtgggtggacgtga  
gccacgaagacctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc  
tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaggcttctatccagcgacatcgccgtgga  
35 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcagggaacgttctctcatgctccgtgatgcatgaggctctgcacaaccactacac  
cagaagagcctctccctgtctccgggtaaatgatctaga

#### Amino acid sequence

40 mdfqvqifsfllisasviiargqivlsqspailsaspekvmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrpqglewigaiypngdtsynqkfkgtatltvdksstaymqlssltedsavfyfcarvvyysnsywy  
fdvwgtgtvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrnpvctcvvdvshedpevkfnwyvdgvev  
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitc  
45 lvkgfypsdiavewesngqpennyktpvpldsdsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispk

### 32. 2H7 scFv VH L11S (CSS-S)H T256N CH2 WCH3

Nucleotide sequence:

5 aagcttgccgcatggaatttcaagtgcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatacttgccagggccagctcaagtgaagttacatgcactgg  
taccagcagaagccagatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggaccttactctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagcttggggctgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
10 ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcaggagcccaatcttgtacaaaactcacatccccaccgtcctcagcacctgaactcctgggggga  
ccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccgaacctgaggtcacatgcgtgggtggagcgtga  
15 gccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca  
20 agctcaccgtggacaagagcaggtggcagcagggaacgtcttctcatgctcctgtagcatgaggctctgcacaaccactacacg  
cagaagagcctctccctgtctccggtaaatgatctaga

Amino acid sequence

25 mdfvqvifsfllisasviiargqivlsqspailsaspgekvtmtrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveadaatyccqqsfnptpfagtkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy  
fdvwtgtttvssdqepkscdkthtsppssapellggpsvflfppkpkdtlmismpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvylppsrdeltnqvsltc  
lvkgfypsdiavewesngqpennyktppldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

### 33. RTPE/QNAK (255-258) CH2

Nucleotide sequence:

35 cctgaactcctggggggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccagaacgctaaggctacat  
gcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa  
ggagtacaagtgaaggtctccaacaagccctcccagccccatcgagaaaacaatctccaagccaaa

Amino acid sequence

40 pellggpsvflfppkpkdtlmisqnakvtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdw  
lngkeykckvsnkalpapiektiskak

### 34. RTPE/QNAK (255-258)CH2 WCH3

Nucleotide sequence:

45 cctgaactcctggggggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccagaacgctaaggctacat  
gcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa

ggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgaga  
 accacaggtgtacacctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctggtcgaaggcttctatc  
 ccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgctcccgctgctggactccgac  
 ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag  
 5 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

pellggpsvflfppkpkdtlmsiqnakvtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqd  
 lngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltnqvslclvkgfypsdiavewesngqpennyktpvp  
 10 ldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

**35. 2H7 scFv VH L11S (SSS-S)H RTPE/QNAK (255-258)CH2 WCH3**

Nucleotide sequence:

15 aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctc  
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
 gtgggtctgggaccttctactctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggttaac  
 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
 20 tctcaggcttatctacagcagcttggggctgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
 ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatgggtgatact  
 tctacaatcagaagttaagggaagccacactgactgtagacaatactccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
 ggtcaccgtctctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
 25 ccgtcagttctcttccccccaaaacccaaggacaccctcatgatctcccagaacgtaaggtcacatgcgtgggtggagtgga  
 gccacgaagaccctgaggtcaagttaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcgaggagc  
 agtacaacagcacgtaccgtgtgtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc  
 tccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaaggcagccccgagaaccacaggtgtacacctg  
 cccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcgaaggcttctatccagcgacatcgccgtgga  
 30 gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttctctctacagca  
 agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence

35 mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
 sgsqtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
 gytftsynmhwwkqtrqglewigaiypngdtsynqfkkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy  
 fdvwtgtttvtssdqepkssdkthtsppssapellggpsvflfppkpkdtlmsiqnakvtcvvvdvshedpevkfnwyvdg  
 evhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltnqvslc  
 40 lvkgfypsdiavewesngqpennyktpvpldsdsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

**36. 2H7 scFv VH L11S (CSS-S)H RTPE/QNAK (255-258)CH2 WCH3**

Nucleotide sequence:

45 aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctc  
 ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagtacatgcactgg  
 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga

gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggttttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggttaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
5 tctacaatcagaagtcaagggaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
ccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccagaacgctaaggtcacatgcgtgggtgggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagc  
10 agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
ccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaactcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg  
15 cagaagagcctctccctgtctccgggtaaatgatctaga

#### Amino acid sequence

mdfqvqifsfllisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtklekdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
20 gytftsymhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqllslltsedsavyfcarvvysnsywy  
fdvwtgttvtvssdqepkscdkthtsppssapellggpsvflfppkpkdtlmsqnakvtcvvvdvshedpevkfnwyvdg  
vevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrldeltnqvslt  
clvkgyfypsdiavewesngqpennyktpvpvldsdgsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslslspgk

### 25 36. K290Q CH2 region

#### Nucleotide sequence:

cctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacat  
gcgtggtgggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac  
acagccgaggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa  
30 ggagtacaagtgaaggctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaa

#### Amino acid sequence:

pellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdwl  
ngkeykckvsnkalpapiektiskak

35

### 37. K290Q CH2 WCH3

#### Nucleotide sequence:

Cctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcaca  
tgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaaga  
40 cacagccgaggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa  
ggagtacaagtgaaggctccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgaga  
accacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatc  
ccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac  
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag  
45 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

pellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdwl  
ngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltclvkgyfypsdiavewesngqpennykttppvl  
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

5

**38. 2H7 scFv VH L11S (SSS-S)H K290Q CH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
10 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
15 tctacaatcagaagtcaagggaaggccacactgactgtagacaaaatctccagcagcctacatgcagctcagcagcctgac  
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
ccgtcagcttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgagggtcacatgcgtgggtgggtgacgtga  
gccacgaagaccctgaggtcaagttaactggtagctggagcggctggaggtgcataatgccaagacacagccgcgggaggagc  
20 agtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacacg  
25 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfvqqifsfllisasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfagatkleldggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
30 gytftsynmhvkwqtprrglewigaiypngdtsynqfkkgkatltvdksstaymqlssltedsavyfcarvvysnsywy  
fdvwtggtvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktqpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc  
lvkgyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

**39. 2H7 scFv VH L11S (CSS-S)H K290Q CH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgaggccagctcaagtgttaagttacatgcactgg  
40 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtgga  
gtgggtctgggacctcttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagtcaagggaaggccacactgactgtagacaaaatctccagcagcctacatgcagctcagcagcctgac  
45 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttctactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga



ccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtgggtggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacacagccgaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg  
5 ccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
cagaagagcctctccctgtctccgggtaaatgatctaga

- 10 Amino acid sequence:  
mdfqvqifsflisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsymbhwvkqtrprglewigaiypngdtsynqkfkgkatltvdkssstaymqlssltedsavyfcarvvysnsywy  
fdvwtgtttvtssdqepkscdkthtspssapellggpsvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgv  
15 evhnaktqpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeitknqvsitc  
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgk

#### 40. A339PCH2

Nucleotide sequence:

- 20 cctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacat  
gcgtgggtgggtgacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa  
ggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaaccacaaa

- 25 Amino acid sequence:  
pellggpsvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdw  
lngkeykckvsnkalpapietiskpk

#### 30 41. A339P CH2 WCH3

Nucleotide sequence:

- cctgaactcctggggggaccgtcagttcttcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacat  
gcgtgggtgggtgacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa  
35 ggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctcaaaccacaaagggcagccccgaga  
accacaggtgtacacctgccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatc  
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac  
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag  
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

- 40 Amino acid sequence:  
pellggpsvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgvevhnaktqpreeqynstyrvsvltvlhqdw  
lngkeykckvsnkalpapietiskpkgpprepqvytlppsrdeitknqvsitclvkgfypsdiavewesngqpennyktpv  
45 dsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgk

#### 42. 2H7 scFv VHL11S (SSS-S)H A339P CH2 WCH3

Nucleotide sequence:

aagcttgccgcatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctctgcttcagttggca  
5 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
10 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
ccgtcagttcttcttccccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgggtgggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
15 tccaacaaagccctcccagccccatcgagaaaacaatctccaaacccaaagggcagccccgagaaccacaggtgtacacctgc  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcgaaggcttctatcccagcgacatcgccgtggag  
tgaggagcaatggcgagccgggagaacaactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaa  
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaacctacacgc  
agaagagcctctcctgtctccggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveadaatyycqqwsfnpptfagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrpqglewigaiypngdtsynqfkfkgkatltvdkssstaymqlssltedsavyfcarvvyyysnywy  
25 fdvwtgtttvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvvsvltvlhqdwlngkeyckvsnkalpapiektiskpkqpprepqvylppsrldeltnqvsltc  
lvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispkg

### 30 43. 2H7 scFv VHL11S (CSS-S)H A339P CH2 WCH3

Nucleotide sequence:

aagcttgccgcatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctctgcttcagttggca  
35 gtgggtctgggaccttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
40 atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaactcttactggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
ccgtcagttcttcttccccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgggtgggacgtga  
gccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
45 tccaacaaagccctcccagccccatcgagaaaacaatctccaaacccaaagggcagccccgagaaccacaggtgtacacctgc  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcgaaggcttctatcccagcgacatcgccgtggag

tgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaa  
gctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc  
agaagagcctctccctgtctccgggtaaatgatctaga

5 Amino acid sequence:

mdfqvqifsflisaviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypslnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrpqrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltssedsavyfcarvvysnsywy  
fdvwgtgtvtvssdqepkscdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv  
10 evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskpkqprepqvytlppsrdeltknqvsltc  
lvkgfyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispk

44. G28-1VH

15 Nucleotide sequence:

gcgggtccagctgcagcagcttggaacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttactcattca  
ctggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtggattggaaatattgatccttattatggttggtactacct  
acaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctg  
aggactctgcagcttattactgtgcaagatcggtcggccctatggactactgggggtcaaggaacctcagtcaccgtctctctgatcag

20

Amino acid sequence:

avqlqqsgpelekp gasvkisckasgysftgynmnwvkqnnngkslewnidpyyggttynrkfkkgkatltvdkssstaymq  
lksltssedsavyycarsvgpmdywgqgtsvtvssdq

25 45. G28-1VL

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgcttggtat  
cagcagaaacagggaaaatctcctcagctcctggtctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg  
30 gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataatccgtg  
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtggcgccggatcgtca

Amino acid sequence:

mvstaqlflglllwlgtgrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqkspqllvsfaktlaegvpsrfsrgsgs  
35 gtqfslkisslqpedsqsyfcqhhsdnptwfgggteleikggggsgggsgggss

46. G28-1 scFv

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgcttggtat  
cagcagaaacagggaaaatctcctcagctcctggtctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg  
gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatcattccgataatccgtg  
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtggcgccggatcgtcag  
45 cgggtccagctgcagcagcttggaacctgagctggaaaagcctggcgcttcagtgaagatttcctgcaaggcttctggttactcattcact  
ggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtggattggaaatattgatccttattatggttggtactacctac

aaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga  
ggactctgcagctattactgtgcaagatcggcggccctatggactactggggtaaggaacctcagtcaccgtctcttctgatcag

Amino acid sequence:

5 mvstaqflglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfs gsgs  
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggteleikgggsgggsgggssavqlqqsgpelekp gasvkiskasgys  
ftgynmnwvkqnnkslewnidpyyggtynrkfk gkatltvdkssstaymqlksltsedsavyy carsvgpmdywggq  
tsvtvssdq

10

#### 47. G28-1 VHL11S

Nucleotide sequence:

gcgggtccagctgcagcagcttgacgtcggaagcctggcgcttcagtgaa gatttctgcaaggcttctggttactcattca  
ctggctacaatatgaactgggtgaagcagaataatggaagagccttgagtgattggaaatattgatccttattatgggtggtactac  
15 acaaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctg  
aggactctgcagctattactgtgcaagatcggcggccctatggactactggggtaaggaacctcagtcaccgtctcttctgatcag

Amino acid sequence:

20 avqlqqsgpesekpgasvkiskasgysftgynmnwvkqnnkslewnidpyyggtynrkfk gkatltvdkssstaym  
qlksltsedsavyy carsvgpmdywggqtsvtvssdq

#### 48. G28-1 VHL11S scFv

Nucleotide sequence:

aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacagg tggcagatgtgacatccagatgactca  
gtctccagctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagt gaaaatgtttacagttattggcttggtat  
cagcagaaaacagggaaaatctcctcagctcctggctctctttgcaaaaaccttagcagaaggtgtgccatcaagg ttcagtggcagtg  
gatcaggcacacagtttctctgaagatcagcagcctgcagcctgaagattctggaagtattttctgtcaacatcattccgataatccgtg  
30 gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggcggcg ggcggatcgtcag  
cgggtccagctgcagcagcttgacgtgagtcggaagcctggcgcttcagtgaa gatttctgcaaggcttctggttactcattcact  
ggctacaatatgaactgggtgaagcagaataatggaagagccttgagtgattggaaatattgatccttattatgggtggtactacctac  
aaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga  
ggactctgcagctattactgtgcaagatcggcggccctatggactactggggtaaggaacctcagtcaccgtctcttctgatcag  
35

Amino acid sequence:

mvstaqflglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfs gsgs  
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggteleikgggsgggsgggssavqlqqsgpesekpgasvkiskasgys  
ftgynmnwvkqnnkslewnidpyyggtynrkfk gkatltvdkssstaymqlksltsedsavyy carsvgpmdywggq  
40 tsvtvssdq

#### 49. G28-1 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

45 aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacagg tggcagatgtgacatccagatgactca  
gtctccagctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagt gaaaatgtttacagttattggcttggtat

cagcagaaacagggaaaatctcctcagctcctggtctcttttgcaaaaccttagcagaaggtgtgcatcaaggttcagtggcagtg  
 gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatattccgataatccgtg  
 gacgttcggtggagggaccgaactggagatcaaaggtggcgggtggctcggcggtggtgggtcgggtggcgccggatcgtcag  
 cggctccagctgcagcagctctggacctgagctggaaaagcctggcgcttcagtgaaagatttctgcaaggcttctggttactcattcact  
 5 ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatggtggtactacctac  
 aaccgggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctga  
 ggactctgcagctctattactgtgcaagatcggcggcctatggactactgggtcaaggaacctcagtcaccgtctcttctgatcatg  
 atcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaactcctggggggaccgtcagcttctccttctc  
 ccccaaaaaccaaggacacctcatgatctccggacccctgaggtcacatgcgtgggtgggtggacgtgagccacgaagacctg  
 10 aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagcagtacaacagcacgt  
 accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctc  
 ccagcccccacagagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtgat  
 gagctgaccaagaaccaggtcagcctgacctgcctggcctcaaggttctatcccagcgacatcgccgtggagtgggagagcaatg  
 ggcagccgggagaacaactacaagaccacgctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggac  
 15 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
 cctgtctccgggtaaatgatctaga

Amino acid sequence:

mvstaqlfllllwtggcdiqmtqspaslsasvgetvtitrtsenvysylawyyqqkqgkspqllvsfaktlaegvpsrfsrgsgs  
 20 gtqfslkisslqpedsgsyfcqhhdnpwtfgggteleikggggsgggsgggssavqlqqsgpelekpgasvkiskcasgys  
 ftgynmnwvkqnnkslewignidpyyggtytnrkfkkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
 tsvtvssdhdepkssdkthtspssapellggspsvflfppkpkdtlmisrtpcvvvdvshedpevkfnwyvdgvevhna  
 ktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvslclvkqf  
 ypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk  
 25

## 50. G28-1 scFv IgAW H IgG1WCH2 WCH3

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
 30 gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggttggtat  
 cagcagaaacagggaaaatctcctcagctcctggtctcttttgcaaaaccttagcagaaggtgtgcatcaaggttcagtggcagtg  
 gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtcaacatattccgataatccgtg  
 gacgttcggtggagggaccgaactggagatcaaaggtggcgggtggctcggcggtggtgggtcgggtggcgccggatcgtcag  
 cggctccagctgcagcagctctggacctgagctggaaaagcctggcgcttcagtgaaagatttctgcaaggcttctggttactcattcact  
 35 ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatggtggtactacctac  
 aaccgggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctga  
 ggactctgcagctctattactgtgcaagatcggcggcctatggactactgggtcaaggaacctcagtcaccgtctcttctgatcagc  
 cagttccctcaactccactacccatctccctcaactccactacccatctccctcatgcccacctgaactcctggggggaccgtca  
 gtcttctcttcccccaaaaaccaaggacacctcatgatctcccgacccctgaggtcacatgcgtgggtgggtgagcgtgagccac  
 40 gaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggaggagcagtac  
 aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaa  
 caaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccc  
 atccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaggttctatcccagcgacatcgccgtggagtggg  
 agagcaatgggcagccggagaacaactacaagaccacgctcccggtgctggactccgacggctccttcttctctacagcaagctc  
 45 accgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcaga  
 agagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs  
gtqfslkisslqpedsgsyfcqhhsdnpwtfgggteleikggggsgggsgggssavqlqsgpelekpgasvkisckasgys  
5 ftgynmnwvkqnnkslewnidpyyggtynrkfkkgkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
tsvtvssdqvpstptpspstpsscapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvev  
hnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltclv  
kgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

10 51. **G28-1 scFv VHL11S (SSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
gtctccagctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttattggcttggtat  
cagcagaaacagggaaaatctcctcagctcctggtctcttttgcacaaaccttagcagaaggtgtccatcaaggttcagtggcagtg  
15 gatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagtattttctgcaacatcattccgataatccgtg  
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtggcgccggtatcgtcag  
cgggtccagctgcagcagcttgacgtgagtcggaaaagcctggcgttcagtggaagatttctgcaaggcttctggttactcattcact  
gggtacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttggaatatgatccttattatgggtgtactacctac  
aaccgggaagtcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagctgacatctga  
20 ggactctgcagctattactgtgcaagatcggcggcctatggactactgggtcaaggaacctcagtcaccgtctcttctgatcagg  
agcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtcagttctcttctcccccc  
aaaaccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtc  
aagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt  
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaggctccaacaaagccctcccagc  
25 ccccatcgagaaaacaatctccaaagcgaaggcagccccgagaaccacaggtgtacaccctcccccatccgggatgagct  
gaccaagaaccaggtcagcctgacctgcctggctcaaaggtcttatccagcgacatcgccgtggagtgaggagcaatgggcag  
ccggagaacaactacaagaccacgctccccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagag  
caggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtc  
tccgggtaaatgatctaga

30

Amino acid sequence:

mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs  
gtqfslkisslqpedsgsyfcqhhsdnpwtfgggteleikggggsgggsgggssavqlqsgpesekpgasvkisckasgys  
35 ftgynmnwvkqnnkslewnidpyyggtynrkfkkgkatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
tsvtvssdhdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhna  
ktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsltclvkgf  
ypsdiaavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

40 52. **G28-1 scFv VHL11S (CSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
gtctccagctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttattggcttggtat  
cagcagaaacagggaaaatctcctcagctcctggtctcttttgcacaaaccttagcagaaggtgtccatcaaggttcagtggcagtg  
gatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagtattttctgcaacatcattccgataatccgtg  
45 gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtcgggtggcgccggtatcgtcag  
cgggtccagctgcagcagcttgacgtgagtcggaaaagcctggcgttcagtggaagatttctgcaaggcttctggttactcattcact

ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatgggtgtactacctac  
 aaccgggaagtcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga  
 ggactctgcagtctattactgtgcaagatcggcggccctatggactactggggtcaaggaacctcagtcaccgtctcttctgatcagg  
 agcccaaatcttgtgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggaccgtcagctcttcttcccccc  
 5 aaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggacgtgagccacgaagacctgaggtc  
 aagtcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt  
 gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagc  
 ccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagct  
 gaccaagaaccaggtcagcctgacctgcctgggtcaaaggtcttatccagcgacatcgccgtggagtgggagagcaatgggcag  
 10 ccggagaacaactacaagaccacgcctccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagag  
 caggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtc  
 tccggtaaatgatctaga

Amino acid sequence:

15 mvstaqlglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghs  
 gtqfslkisslqpedsgsyfcqhhsdnpwtfgggteleikggggsgggsgggssavqlqsgpeseekpgasvkiskcasgys  
 ftgynmnwvkqnnkslewnidpyyggtynrkfkkatltvdksstaymqlksltsedsavyycarsvgpmidywggg  
 tsvtvssdqepksckdhtspssapellggsfvflppkpkdtlmisrtevtcvvdvshedpevkfnwyvdgvevhnakt  
 kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitclvkgyfyp  
 20 sdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhnhytqkslsispkg

### 53. G28-1 scFv VH L11S (CSC-S)H WCH2 WCH3

Nucleotide sequence:

25 aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
 gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggcttggtat  
 cagcagaaacagggaaaatcctcagctcctggtctcttttgcaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg  
 gatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagtatttctgtaacatcattccgataatccgtg  
 gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtggcggcgatcgtcag  
 30 cggtcagctgcagcagcttgacctgagtcggaagacctggcgcttcagtgaaagatttctgcaaggcttctggttactcattcact  
 ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggattggaaatattgatccttattatgggtgtactacctac  
 aaccgggaagtcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga  
 ggactctgcagtctattactgtgcaagatcggcggccctatggactactggggtcaaggaacctcagtcaccgtctcttctgatcagg  
 agcccaaatcttgtgacaaaactcacacatctccaccgtgtcagcacctgaactcctgggtggaccgtcagcttcttcttccccca  
 35 aaaccaagagacacctcatgatctccggaccctgaggtcacatgcgtgggtggacgtgagccacgaagacctgaggtca  
 agttcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtg  
 ggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagcc  
 cccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctg  
 accaagaaccaggtcagcctgacctgcctgggtcaaaggtcttatccaagcgacatcgccgtggagtgggagagcaatgggcagc  
 40 cggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttcttctctacagcaagctcaccgtggacaagagc  
 aggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtct  
 ccgggtaaatgatctaga

Amino acid sequence:

45 mvstaqlglillwtggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsghs  
 gtqfslkisslqpedsgsyfcqhhsdnpwtfgggteleikggggsgggsgggssavqlqsgpeseekpgasvkiskcasgys

ftgynmnwvkqnnkslewignidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
tsvtvssdqepkscdkthtspcsapellggpsvflfppkpdltlmisrtpetvvcvvdvshedpevkfnwyvdgvevhnakt  
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsitclvkgyfyp  
sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

5

#### 54. G28-1 scFv VH L11S (SSC-P)H WCH2 WCH3

Nucleotide sequence:

10 aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttggttggtat  
cagcagaaacagggaatatctctcagctcctgtctcttttgcacaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg  
gatcaggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagtattttctgcaacatcattccgataatccgtg  
gacgttcggtggagggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtggcgccggtatcag  
cgggtccagctgcagcagcttgacactgagtcggaaaagcctggcgcttcagtggaagatttctgcaaggcttctggttactcattcact  
15 ggctacaatatgaactgggtgaagcagaataatggaaagagccttgagtggttggaatatgatccttatttggtgtactacctac  
aaccggaagttcaagggcaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagcttgacatctga  
ggactctgcagcttattactgtgaagatcggcggcctatggactactgggtgcaaggaacctcagtcaccgtctcttctgatcagg  
agcccaaatcttctgacaaaactcacacatccccaccgtgccagcacctgaactcctggggggaccgtcagcttctcttcccccc  
aaaaccaagacacacctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtc  
20 aagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtagaacagcacgtaccgt  
gtggtcagcgtctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctccagc  
ccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatcccggtgatgact  
gaccaagaaccaggtcagcctgacctgcctgggtcaaaggtcttatccagcgacatcgccgtggagtgaggagcaatgggcag  
ccggagaacaactacaagaccacgctcccgtgctggactccgacggctcttctctctacagcaagtcaccgtggacaagag  
25 caggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggtctgcacaaccactacacgcagaagagcctctccctgtc  
tccgggtaaatgatctaga

Amino acid sequence:

30 mvstaqlflglilwtlgtgrcdiqmtqspaslsasvgetvtitrtstenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs  
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggteleikggggsgggsgggssavqlqqsgpesekpgasvkiskcasgys  
ftgynmnwvkqnnkslewignidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
tsvtvssdqepksscdkthtspcpapellggpsvflfppkpdltlmisrtpetvvcvvdvshedpevkfnwyvdgvevhnakt  
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsitclvkgyfyp  
sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

35

#### II. 54. HCTLA4 HIGG1 (SSS-S)H P238SCH2 WCH3

Nucleotide sequence:

40 atggcttgcccttgatttcagcggcacaaggtcagctgaacctggctgccaggacctggcctgcactctctgtttttcttcttcat  
ccctgtcttctgcaagcaatgcacgtggcccagcctgctgtgtgactggccagcagccaggcatcgccagcttctgtgtgagtat  
gcatctccaggcaaaagccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcgggcaacct  
acatgacggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctactatccaagg  
actgagggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggaacggaa  
cccagatttatgtaattgatccagaaccgtgccagattctgatcaacccaaatcttctgacaaaactcacacatccccaccgtctcag  
cacctgaactcctggggggatcgtcagcttctcttccccccaaaaccaaggacacctcatgatctccggacccttgaggtcac  
45 atcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag  
acaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggca



aggagtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgag  
aaccacaggtgtacaccctgccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctat  
cccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccga  
cggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcata  
5 ggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

Amino acid sequence:

maclgfqrhkaqlnlaartwpctllfflfpvfckamhvaqpavvlassrgiasfvceyaspgkatevrvtlrqadsqvtevaa  
tymtgneltflddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyyidpepcpdsdpkssdkhts  
10 ppssapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlh  
qdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdektknqvsitclvkgfypsdiavewesngqpennykt  
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslsispk\*

## 55. FC2-2 VL

15 Nucleotide sequence:

gttgtaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt  
cacagctccatctccttagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagcctttatataatcacaatca  
aaagaactacttgccctgggtaccagcagataaccaggggcagctcctaaactgctgattactgggcatccactagggaaatctggggtc  
cctgatcgcttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg  
20 tcagcaatattatactatctctccacgttcggaggtggcaccaagctggaaataaaagggtggcggtggctcgggcgggtgggtgggtc  
gggtggcgccgggagctcg

Amino acid sequence:

mdsqavlmllllwvsgtcdvmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres  
25 gvpdrftgsgsgtdflitirvkaedlavyyccqyytypptfgggtkleikggggsgggsgggss

## 56. FC2-2VH

Nucleotide sequence:

Gggagctcgcaggtgcagttgaaggagtcaggacctggcctgggtggcgccctcacagagcctgtccatcacatgcaccgtctcag  
30 ggttctcattaaccgtctatggtgtaactgggttcggcagcctccaggaaagggtctggactggctgggaatgatatggggatgg  
aagcacagactataattcagctctcaatccagactgagcatcagtaaggacaactccaagagccaagttttctaaaaatggacagtc  
tacaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggcgaaggaaacctc  
agtcaccgtctcctctgatcag

35 Amino acid sequence:

gssqvqlkesgpglvapsqslsitctvsgfsltygvnvwvrpppgkglwdlwmigwdgstdynsalksriskdnksqvflk  
mdslqtdtdaryycardhygthyamdywgqgtsvtvssdq

## 40 57. FC2-2scFv

Nucleotide sequence:

gttgtaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt  
cacagctccatctccttagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagcctttatataatcacaatca  
aaagaactacttgccctgggtaccagcagataaccaggggcagctcctaaactgctgattactgggcatccactagggaaatctggggtc  
45 cctgatcgcttcacaggcagtgatctgggacagattcactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg  
tcagcaatattatactatctctccacgttcggaggtggcaccaagctggaaataaaagggtggcggtggctcgggcgggtgggtgggtc

gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggcctggcggcgcctcacagagcctgtccatcacatgc  
accgtctcaggggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatg  
gggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaa  
atggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc  
5 aaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:

mdsqaqvlmllllwwsgtcgdivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres  
gvpdrftgsgsgtdflitirvkaedlavyyccqyytypptfgggtkleikgggsgggsgggssqvqlkesgpglvapsql  
10 sitctvsgfsltyvgvnnwvrppgkglwlgmiwgdgstdynsalksrslskdnksqvflkmdslqddtaryycardhygt  
hyamdywgqgtsvtvssdq

### 58. FC2-2 VHL11S

15 Nucleotide sequence:

gggagctctcaggtgcagttgaaggagtcaggacctggctcggcggcgcctcacagagcctgtccatcacatgcaccgtctcagg  
gttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatggggtgatgga  
agcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaaatggacagctt  
acaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtcaaggaacctca  
20 gtcaccgtctcctctgatcag

Amino acid sequence:

(gss)qvqlkesgpgsvapsqlsitctvsgfsltyvgvnnwvrppgkglwlgmiwgdgstdynsalksrslskdnksqvfl  
kmdslqddtaryycardhygthyamdywgqgtsvtvssdq  
25

### 59. FC2-2 VH L11S scFv

Nucleotide sequence:

gttgtaagcttgccgccatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt  
30 cacagctcctccctcctagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagcctttatataatcacatca  
aaagaactacttgccctgggtaccagcagataaccagggcagctctcctaaactgctgatttactgggcatccactagggaatctggggtc  
cctgatcgcttcacaggcagtggtatctgggacagatttactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg  
tcagcaatattatacctatcctccacgttcggaggtggcaccaagctggaaataaaagggtggcgggtggctcgggagggtgggtc  
gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggctcgggtggcgcctcacagagcctgtccatcacatgc  
35 accgtctcaggggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatg  
gggtgatggaagcacagactataattcagctctcaaatccagactgagcatcagtaaggacaactccaagagccaagtttcttaaaa  
atggacagtctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc  
aaggaacctcagtcaccgtctcctctgatcag

40 Amino acid sequence:

Mdsqaqvlmllllwwsgtcgdivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres  
gvpdrftgsgsgtdflitirvkaedlavyyccqyytypptfgggtkleikgggsgggsgggssqvqlkesgpgsvapsql  
sitctvsgfsltyvgvnnwvrppgkglwlgmiwgdgstdynsalksrslskdnksqvflkmdslqddtaryycardhygt  
hyamdywgqgtsvtvssdq  
45

### 60. FC2-2 (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgcatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt  
cacagctccatccctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagcctttatataatcacatca  
aaagaactacttggcctgggtaccagcagataaccaggggcagtcctctaaactgctgatttactgggcatccactagggaatctggggtc  
5 cctgatcgcttcacaggcagtgatctgggacagatttactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg  
tcagcaatattatacctatcctccacgttcggaggtggcaccagctggaaataaaagggtggcgggtggctcgggcgggtgggtgggtc  
gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggcctgggtggcggcctcacagagcctgtccatcacatgc  
accgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatg  
gggtgatggaagcacagactataattcagctctcaatccagactgagcatcagtaaggacaactccaagagccaagttttcttaaaa  
10 atggacagctctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc  
aaggaaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaact  
cctgggtggaccgtcagcttctcttcccccaaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtg  
gtggacgtgagccacgaagacctgaggtcaagttcaactgtgtacgtggacggcgtggaggtgcataatgccaaagacaaagccgc  
gggaggagcagtagacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa  
15 gtgcaaggcttccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt  
gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgtaaaggcttctatccaagcgaca  
tcgccgtggagtgggagagcaatgggcagccgggagaacaaactacaagaccacgctcctcgtgctggactccgacggctccttct  
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca  
accactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdsqavqlmlllwvsgtcgdivmsqspsslavsvgekvsmckssqsllynhnqnknylawyqqipgqspklliywastres  
gvpdrftgsgsgtdflitirvkaedlavyycqqyyttypptfgggtkleikgggsgggsgggsgssvqlkesgpglvapsql  
sitctvsgfltyvgvwnvrppgkglwdlwmigwdgstdynsalksrslskdnskqvflkmdslqtdtdaryycardhygt  
25 hyamdywgqgtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsirtpevtcvvdvshedpevkfnwy  
vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltkn  
qvsltclvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp  
gk

### 30 61. FC2-2 VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttgccgcatggattcacaggcccaggttcttatgttactgctgctatgggtatctggtacctgtggggacattgtgatgt  
cacagctccatccctccctagctgtgtcagttggagagaaggtttctatgagctgcaagtccagtcagagcctttatataatcacatca  
aaagaactacttggcctgggtaccagcagataaccaggggcagtcctctaaactgctgatttactgggcatccactagggaatctggggtc  
35 cctgatcgcttcacaggcagtgatctgggacagatttactctcaccatcagcagagtgaaggctgaagacctggcagtttattactg  
tcagcaatattatacctatcctccacgttcggaggtggcaccagctggaaataaaagggtggcgggtggctcgggcgggtgggtgggtc  
gggtggcggcgggagctctcaggtgcagttgaaggagtcaggacctggctcgggtggcggcctcacagagcctgtccatcacatgc  
accgtctcagggttctcattaaccgtctatggtgttaactgggttcgccagcctccaggaaagggtctggactggctgggaatgatatg  
gggtgatggaagcacagactataattcagctctcaatccagactgagcatcagtaaggacaactccaagagccaagttttcttaaaa  
40 atggacagctctacaaactgatgacacagccaggtactactgtgccagagatcactatggtaccactatgctatggactactggggtc  
aaggaaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaact  
cctgggtggaccgtcagcttctcttcccccaaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtg  
gtggacgtgagccacgaagacctgaggtcaagttcaactgtgtacgtggacggcgtggaggtgcataatgccaaagacaaagccgc  
gggaggagcagtagacaacagcacgtaccgtgtgtgcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaa  
45 gtgcaaggcttccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt  
gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgtaaaggcttctatccaagcgaca

tcgccgtggagtgaggagcaatgggcagccggagaaactacaagaccacgcctcccgctgctggactccgacggctccttctt  
cctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcaca  
accactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5 Amino acid sequence:

mdsqaqvlmllllwwsvgtcgdivmsqspsslavsvgekvsmsckssqsllynhnqknylawyqqipgqspklliywastres  
gvpdrftgsgsgtdftltisrvkaedlavyyccqyytypptfggggkleikggggsgggsgggssqvqlkesgpgsvapsqsl  
sitctvsgfsltygvnwvrqppgkgldwlgmiwgdgstdynsalksrlsiskdnsksqvflkmdslqddtaryycardhygt  
hyamdywgqgtsvtvssdqepkssdkhtsppsapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
10 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltkn  
qvsltclvkgyfypsdiavewesngqpennyktpplvdsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp  
gk

**62. UCHL-1 VH**

15 Nucleotide sequence:

atgggcaggcttacttctcattcctgctactgattgttctgcataatgcctctcccagattactctgaaagagcttggccctgggatcttg  
cagccctcccagaccctcagctgacttgttcttctctgggtttcactgaccactatggtataggagtaggttgattcgtcagcctcc  
aggggaagggtctggagtggtgacacacatttggtggaatgataataagtactataacacagccctgaggagccggctcacaatctc  
caaggattcctccaacaaccaagtactcctcaagatcgccaatgtggacactgcagataccgccacatactactgtctctacggctac  
20 acttactggggccaagggactctgtgactgtctctgca

Amino acid sequence:

mgrltssflilivpayvlsqitlkesgpgilqpsqtsltsfsgfsltyigvgwirppgkglewlthiwwndnkyyntalrsrli  
skdssnnqvllkianvdtadtatyyclygytywggtlvtvsa  
25

**63. UCHL-1 VL**

Nucleotide sequence:

atgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgacccaaactccactctccctgcc  
tgtcagcttggagatcaggcctccatctcttcagatctagtcagagcctctttacagtaatggaaacacctattacattggtacctgc  
30 agaagccaggccagctctccaaaactcctgatctacaaactttccaaccgattttctgggggtcccagacaggttcagtggcagtgatc  
agggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaaagtacacatgttccgtgga  
cgttcggtggaggcaccaagctggaaatcaaaa

Amino acid sequence:

35 mklpvrllvlmfwpasisdvmtqtplslpvsldqasiscrssqsllysnngntylhwylqkpgqspklliyklsnrfsgvpdrf  
sgsgsgtdftlkisrveadlgvyfcsqsthvpwtfggggleik

**64. UCHL-1 scFv**

40 Nucleotide sequence:

ggtgtaagcttgccgccatgaagttgcctgttaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca  
aactccactctccctgctgctcagcttggagatcaggcctccatctcttcagatctagtcagagcctctttacagtaatggaaacacc  
tatttacattggtacctgcagaagccaggccagctctccaaaactcctgatctacaaactttccaaccgattttctgggggtcccagacagg  
ttcagtggcagtgatcagggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaaa  
45 gtacacatgttccgtggacgttcggtggaggaccaagctggaaatcaaatggcgggtggctcgggcgggtggatctggagg  
aggtgggagctctcagattactctgaaagagtctggccctgggactctgcagccctcccagaccctcagctgacttgttcttctctgg

gtttcactgaccacttatggtataggagtaggttgattcgtcagcctccaggaagggtctggagtggctgacacacatttggtgga  
atgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgc  
caatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgctg  
atca

5

Amino acid sequence:

mklpvrllvmlfwipasisdvmtqtplslpvsldqasiscrssqsllysnngntylhwylqkpgqspklliyklsnrfsqvpdrf  
sgsgsgtdftlkisrveaedlgvyfcsqsthvpwtfgggkldggsgggsgggssqitlkesgpgilqpsqtlsltsfsgf  
sltygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllkianvdtadtatyyclygytywgqgtlvtvs  
ad

10

#### 65. UCHL-1 VH I11SL12S

Nucleotide sequence:

15 gggagctctcagattactctgaaagagtctggccctgggatcttgcagccctcccagaccctcagctgactgttcttctctgggttt  
cactgaccacttatggtataggagtaggttgattcgtcagcctccaggaagggtctggagtggctgacacacatttggtggaatga  
taataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgccaat  
gtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgctgatca

20

Amino acid sequence:

(gss)qitlkesgpgssqpsqtlsltsfsgfsltygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllki  
anvdtadtatyyclygytywgqgtlvtvsad

#### 25 66. UCHL-1 scFv VH L11S

Nucleotide sequence:

gttgtaagcttccgccatgaagttgcctgtaggctgttggtgctgatgttctggattcctgctccatcagtgatgttgatgaccca  
aactccactctccctgcctgtcagcttggagatcaggcctccatcttgcagatctagtcagagcctctttacagtaatggaacacc  
tatttacattggtacctgcagaagccaggccagctccaaaactcctgatctacaaacttccaaccgattttctgggggtcccagacagg  
30 ttcagtggcagtgatcaggacagatttcacactcaagatcagcagagtgaggctgaggatctgggagttatttctgctctcaaa  
gtacacatgttcctgtgacgttcgggtggagggaccaagctgaaatcaaagatggcgggtggctcgggcgggtggtgatctggagg  
aggtgggagctctcagattactctgaaagagtctggccctgggagctcccagccctcccagaccctcagctgactgttcttctctg  
ggtttctactgaccacttatggtataggagtaggttgattcgtcagcctccaggaagggtctggagtggctgacacacatttggtgg  
aatgataataagtactataacacagccctgaggagccggctcacaatctccaaggattcctccaacaaccaagtactcctcaagatcg  
35 ccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgct  
gatca

Amino acid sequence:

40 mklpvrllvmlfwipasisdvmtqtplslpvsldqasiscrssqsllysnngntylhwylqkpgqspklliyklsnrfsqvpdrf  
sgsgsgtdftlkisrveaedlgvyfcsqsthvpwtfgggkldggsgggsgggssqitlkesgpgssqpsqtlsltsfsgf  
sltygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllkianvdtadtatyyclygytywgqgtlvtvs  
ad

#### 45 67. UCHL-1 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

gttgtaagcttccgccatgaagttgcctgtaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca  
aactccactctccctgcctgtcagcttggagatcaggcctccatctcttcagatctagtcagagccttcttacagtaatggaacacc  
tatttacattggtacctgcagaagccaggccagctccaaaactcctgatctacaaactttccaaccgattttctggggtcccagacagg  
5 ttcagtggcagtggtacaggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaa  
gtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaatggcggtggctcgggcgggtggtggatctggagg  
aggtgggagctctcagattactctgaaagagtctggccctgggatcttcagccctcccagaccctcagctctgacttgttcttctctgg  
gttttactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacacatttgggtga  
atgataataagtactataacacagccctgaggagccggtcacaatctccaaggattcctccaacaaccaagtactcctcaagatcgc  
caatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgctg  
10 atcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggtggaccgtcagcttctctctc  
ccccaaaacccaaggacaccctcatgatctcccgaccctgagggtcacatgcgtggtggtggacgtgagccacgaagaccctg  
aggtcaagttaactggtacgtggacggcgtggagggtgataatgccaagacaaagccgcgggaggagcagtacaacagcacgt  
accgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctc  
ccagcccccatcgagaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatcccgggat  
15 gagctgaccaagaaccaggtcagcctgacctgcctggtaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg  
ggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggac  
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
cctgtctccgggtaaatgatctaga

20 Amino acid sequence:  
mklpvrllvmlfwipasisdvmtqtplslpvsldqasiscrssqsllysnngtylhwyqlkpgqspklllyklsnrfsgvpdrf  
sgsgsgtdflkisirveadlgyvfcsqsthvpwtfgggtkleikdggsgggsgggssqitkesgpilqpsqtslscsfsgf  
slttygigvgwirppgkglewlthiwwndnkyyntalrslrskdssnnqvllkianvdtadtatyyclygytywqggtlvts  
adqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpetcvvvdvshedpevkfnwyvdgvevhnaktkpreeq  
25 ynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvslclvkgyfypsdiave  
wesngqpennyktpvpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslspsgk

# 68. UCHL-1 scFv VHL11S (SSS-S)H WCH2 WCH3

30 Nucleotide sequence:  
gttgtaagcttccgccatgaagttgcctgtaggctgttggtgctgatgttctggattcctgcttccatcagtgatgttgatgaccca  
aactccactctccctgcctgtcagcttggagatcaggcctccatctcttcagatctagtcagagccttcttacagtaatggaacacc  
tatttacattggtacctgcagaagccaggccagctccaaaactcctgatctacaaactttccaaccgattttctggggtcccagacagg  
35 ttcagtggcagtggtacaggacagatttcacactcaagatcagcagagtggaggctgaggatctgggagtttatttctgctctcaa  
gtacacatgttccgtggacgttcggtggaggcaccaagctggaaatcaaatggcggtggctcgggcgggtggtggatctggagg  
aggtgggagctctcagattactctgaaagagtctggccctgggagctcccagccctcccagaccctcagctctgacttgttcttctctg  
ggtttactgaccacttatggtataggagtaggttgattcgtcagcctccagggaagggtctggagtggctgacacacatttgggtg  
aatgataataagtactataacacagccctgaggagccggtcacaatctccaaggattcctccaacaaccaagtactcctcaagatcg  
ccaatgtggacactgcagataccgccacatactactgtctctacggctacacttactggggccaagggactctggctactgtctctgct  
40 gatcaggagcccaaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggtggaccgtcagcttctctctt  
ccccaaaacccaaggacaccctcatgatctcccgaccctgagggtcacatgcgtggtggtggacgtgagccacgaagaccct  
gaggtcaagttcaactggtacgtggacggcgtggagggtgataatgccaagacaaagccgcgggaggagcagtacaacagcacg  
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccct  
cccagccccatcgagaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatcccggga  
45 tgagctgaccaagaaccaggtcagcctgacctgcctggtaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg  
ggcagccgggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggac

aagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
cctgtctccggtaaatagatctagaa

Amino acid sequence:

5 mklpvrlvlmfwipasisdvmtqtplslpvslgdqasiscrssqsllysnngtylhwylqkpgqspklliyklsnrfsqvpdrf  
sgsgsgtdfllkisirveaedlgvyfcsqsthvpwtfgggtkleikdggsgsgsgsgsgsgssqitlkesgpgssqpsqtlstcsfsgf  
slttygigvgwirppgkglewlthiwwndnkyyntalrsrltiskdssnnqvllkianvdtadtatyyclygytywgqgtlvtvs  
adqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpvctcvvdvshedpevkfnwyvdgvevhnaktkpreeq  
10 ynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltclvkgfypsdiave  
wesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

## 69. 5B9 VH

Nucleotide sequence:

15 atggctgtcttggggctgctcttctgcctggtagacatttccaagctgtgtcctatcccaggtgcagctgaagcagtcaggacctggcct  
agtgcagtcctcacagagcctgtccatcacctgcacagtctctggttctcattaactacctatgctgtacactgggttcgccagctctcca  
ggaaagggctggagtggtggagtgatggagtggtggaatcacagactataatgcagcttccatccagactgagcatcacc  
aaggacgattccaagagccaagtttctttaaaatgaacagctgcacacctaataacacagccatttattactgtgccagaaatgggggt  
gataactacccttattactatgctatggactactggggtaaggaacctcagtcaccgtctcctca

20

Amino acid sequence:

mavlgllfclvtfpscvlsqvllkqsgpglvqssqslsitctvsgfslttyavhwvrqspgkglewlgviwsggitdynaafisrlsi  
tkddsksqvffkmnslqpndtaiyyarnngdnyppyyamdywgqgtsvtvss

## 25 70. 5B9 VL

Nucleotide sequence:

atgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgcaggctgcattctcca  
atccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcacttattgtattgtatc  
tgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtccagacaggttcagtagcagtggt  
30 gtcaggaaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgttattactgtgctcaaaatctagaactccgct  
cacgttcggtgctgggaccaagctggagctgaaacgg

Amino acid sequence:

35 mrfsaqllgllvlwipgstadivmtqaafsnpvtlgtssasiscrskslhsngitylywylqkpgqspqlliyqmsnlasgvpdrf  
sssgsgtdfllrisrveaedvgvycaqnlelpltfaggtklelkr

## 71. 5B9scFv

Nucleotide sequence:

Aagcttggcccatgaggttctctgctcagcttctggggctgcttgtgctctggatccctggatccactgcagatattgtgatgacgca  
40 ggctgcattctccaatccagtcacttctggaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcact  
tattgtattgtatctgcagaagccaggccagctcctcagctcctgatttatcagatgtccaacctgcctcaggagtccagacaggt  
tcagtagcagtggttcaggaactgatttcacactgagaatcagcagagtggaggctgaggatgtgggtgttattactgtgctcaaaat  
ctagaactccgctcacgttcggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtcgggtg  
gcggcgggagctctcaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagtc  
45 tctggttctcattaactacatgctgtacactgggttcgccagctcctcaggaaagggctctggagtggtgggagtgatggagtggt  
ggaatcacagactataatgcagcttccatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaaatgaacagtc

ctgcaacctaataacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaa  
ggaacctcagtcaccgtctcctcag

Amino acid sequence:

5 mrfsaqlglglvlpwipgstadivmtqaafsnpvtlgtasiscrskslhsngitylywylqkpgqspqlliyqmsnlasgvpdrf  
sssgsgtdflrisrveadvgyycaqnlelpltfagtklelkrgggsgggsgggssqvqlkqsgpglvqssqslsitctvs  
gflsttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffkmnslqpndtaiyyccarnggdnpyyya  
mdywgggtsvtvss

#### 10 72. 5B9 VH L11S

Nucleotide sequence:

gggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagctctctggg  
ttctcattaactacatgctgtacactgggttcgccagtcctcaggaaagggtctggagtggtgggagtgatggagtggtggaat  
cacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagctctgca  
15 acctaataacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaaggaa  
ctcagtcaccgtctcctcag

Amino acid sequence:

(gss)qvqlkqsgpgsvqssqslsitctvs gflsttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffk  
20 mnslqpndtaiyyccarnggdnpyyyamdywgggtsvtvss

#### 73. 5B9 VH L11S scFv

Nucleotide sequence:

25 aagcttgccgccatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcag  
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta  
tttgattggtatctgcagaagccaggccagtcctcagctcctgattatcagatgtccaacctgcctcaggagtcacagaggttc  
agtagcagtgggtcaggaactgattcacactgagaatcagcagagtgagggtgaggatgtgggtgttttactgtgctcaaaatct  
agaacttccgctcacgttcgggtgctgggaccaagctggagctgaaacgggggtggcgggtggctcgggcgggtgggtgggtcgggtgg  
30 cggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagctct  
ctggtttctcattaactacatgctgtacactgggttcgccagtcctcaggaaagggtctggagtggtgggagtgatggagtggt  
ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacag  
ctgcaacctaataacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggggtcaa  
ggaacctcagtcaccgtctcctcag

35

Amino acid sequence:

mrfsaqlglglvlpwipgstadivmtqaafsnpvtlgtasiscrskslhsngitylywylqkpgqspqlliyqmsnlasgvpdrf  
sssgsgtdflrisrveadvgyycaqnlelpltfagtklelkrgggsgggsgggssqvqlkqsgpgsvqssqslsitctvs  
gflsttyavhwvrqspgkglewlgviwsggitdynaafisrlsitkddsksqvffkmnslqpndtaiyyccarnggdnpyyya  
40 mdywgggtsvtvss

#### 73. 5B9 scFv (SSS-S)H WCH2 WCH3

Nucleotide sequence:

45 aagcttgccgccatgaggttctctgctcagcttctggggctgcttgctctggatccctggatccactgcagatattgtgatgacgcag  
gctgcattctccaatccagtcactcttgaacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta



5 tttgtattggtatctgcagaagccaggccagtcctcagctcctgatttatcagatgtccaaccttgccctcaggagtcacagacaggttc  
 agtagcagtggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct  
 agaactccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggtcgggcgggtggtgggtcgggtgg  
 cggcgggagctctcaggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagcctgtccatcacctgcacagctc  
 10 ctggtttctcattaactacctatgctgtacactgggttcgccagtcctcaggaaagggtctggagtggctgggagtgatatggagtgg  
 ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt  
 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggtcaa  
 ggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaactcct  
 ggggtggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtggt  
 15 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctaccgtctgcaccaggactggctgaatggcaaggagtacaag  
 tgcaaggttccaacaaagccctcccagcccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccaagcgacatc  
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
 20 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
 cactacacgcagaagagcctctccctgtctccgggtaaatgatctagag

Amino acid sequence:

20 mrfsaqlglvlwipgstadivmtqaafsnpvtlgtssisrskslhsngitylywylqkpgqspqlliyqmsnlasgvpdrf  
 ssssgtdflrisrveadvgyycaqnlepltfagtklelkrsgsgsgsgsgssqvqlkqsgpglvqssqslsitctvs  
 gfslltyavhwvrqspgkglewlgviwsggtdynaafisrlsitkddsksqvffkmnslqpndtaiyyarnngdnyppyya  
 mdywqgtsvtvssdqepkssdkthtspssapellggsvglfpkpkdltlmisrtpvtecvvdvshedpevkfnwyvdg  
 vevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsit  
 25 clvkgyfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

#### 74. 5B9 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

30 aagcttgccgccatgaggttctctgctcagcttctggggctgcttctgctctggatccctggatccactgcagatattgtgatgacgcag  
 gctgcattctccaatccagtcactcttggacatcagcttccatctcctgcaggtctagtaagagtctcctacatagtaatggcatcactta  
 tttgtattggtatctgcagaagccaggccagtcctcagctcctgatttatcagatgtccaaccttgccctcaggagtcacagacaggttc  
 agtagcagtggtcaggaactgattcacactgagaatcagcagagtggaggctgaggatgtgggtgtttactgtgctcaaaatct  
 agaactccgctcacgttcggtgctgggaccaagctggagctgaaacggggtggcgggtggtcgggcgggtggtgggtcgggtgg  
 cggcgggagctctcaggtgcagctgaagcagtcaggacctggctcagtcagtcctcacagagcctgtccatcacctgcacagctc  
 ctggtttctcattaactacctatgctgtacactgggttcgccagtcctcaggaaagggtctggagtggctgggagtgatatggagtgg  
 35 ggaatcacagactataatgcagctttcatatccagactgagcatcaccaaggacgattccaagagccaagtttctttaaataaacagt  
 ctgcaacctaatgacacagccatttactgtgccagaaatgggggtgataactacccttattactatgctatggactactgggtcaa  
 ggaacctcagtcaccgtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaactcct  
 ggggtggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtggt  
 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
 40 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctaccgtctgcaccaggactggctgaatggcaaggagtacaag  
 tgcaaggttccaacaaagccctcccagcccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccaagcgacatc  
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
 45 cactacacgcagaagagcctctccctgtctccgggtaaatgatctagag

Amino acid sequence:

mrfsaqlglglvlpwpgstadvmtqaafsnvptlgtasiscrskslhngitylywylqkpgqspqliyqmsnlasgvpdrf  
sssgsgtdflrisrveadvgyycaqnlelpltfagtklelkrggsgggsgggsgssqvqlkqsgpgsvqssqslsitctvs  
gfslttyavhwvrqspgkglewlgviwsggtdynaafisrlsitkddsksqvffkmnslqpndtaiyyarnngdnyppyya  
5 mdywggqtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdg  
vevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsit  
clvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

#### 75. 2H7 scFv (SSS-S)H P238SCH2 WCH3

10 Nucleotide sequence:

aagcttgccgcatggaatttcaagtcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagttaagtacatgcactgg  
taccagcagaagccagatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggacctcttactcttcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
15 ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tcctacaatcagaagttcaagggcaaggccacactgactgtagacaaatctccagcagacacactacatgcagctcagcagcctgac  
atctgaagactctcggtctattctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacagggaccac  
20 ggtcaccgtctcttctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
tcgtcagctcttcttctccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatcggtggtggagctga  
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgaggagc  
agtacaacagcagctaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtaagaagtc  
tccaacaaagccctcccagccccatcgagaaaacaatccaaagccaaagggcagccccgagaaccacaggtgtacaccctg  
25 ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac  
cagaagagcctctccctgtctccgggtaaatgatctaga

30 Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgkvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfagtklelkdggsgggsgggsgssqaylqqsgaelvrpgasvkmckas  
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkgtatltvdksstaymqlssltedsavvfcarvvyysnsywy  
fdvwtgtttvtvssdqepkssdkthtspssapellggssvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgve  
35 vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitcl  
vkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

#### 76. 2H7 scFv VH L11S (SSS-S)H P238SCH2 WCH3

Nucleotide sequence:

40 aagcttgccgcatggaatttcaagtcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcatctccaggggagaaggtcacaatgacttcagggccagctcaagttaagtacatgcactgg  
taccagcagaagccagatcctcccccacccctggatttatgccccatccaacctggcttctggagtcctgctcgttcagtgga  
gtgggtctgggacctcttactcttcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
45 tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact

5  
10

tctacaatcagaagtcaagggaagggcacactgactgtagacaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggctctatttctgtgcaagagtgggtgactatagtaactttactgggtacttcgatgtctggggcacagggaaccac  
ggtcaccgtctctctgatcaggagcccaatcttctgacaaaactcacacatcccaccgtcctcagcacctgaactcctgggggga  
tcgtcagttctcttcccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggtagtga  
gccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgcatgaggctctgcacaaccactacagc  
cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvmtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveadaatyycqqwsfnpptfgagtklelkdggsggggsggggssqaylqqsgaesvrpgasvkmsckas  
gytftsymbhvwkqtprrglewigaiypngdtsynqkfkakatltvdkssstaymqlssltedsavyfcarvvyyysnsywy  
fdvwgtgtvtvssdqepkssdkthtspssapellgsssvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgve  
vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsitcl  
vkgfypsdiavewesnggpennkykttppvldsdgsffllyskltvdksrwqqgnvfscsvmhhealhnhytqkslslspgk

**77. 2H7 scFv IgA WH WCH2 WCH3 + J Chain**

Nucleotide sequence:

2H7 scFv IgA WH WCH2 WCH3

25 aagcttgccgcatggaatttcaagtcagattttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctcagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgcagggccagctcaagtgtaatgtacatgcactgg  
taccagcagaagccaggatcctccccc aaacctggatttatgccccatccaacctggcttctggagtcctctgctcgttcagtgga  
gtgggtctgggaccttactctctcacaaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtggaagttaaac  
ccaccacgttcgggtctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc  
tctcaggttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagatgtcctgcaaggcttctggctacacat  
30 ttaccagttacaatatgactgggtaaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
tctacaatcagaagttaaggggcaaggccacactgactgtagacaaatctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcgggtctatttctgtgcaagagtgggtgactatagtaactttactggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcagccagttccctcaactccacctaccccattctccctcaactccacctaccccattctccctcatgctgccac  
ccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag  
35 agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgacctctgtggctg  
ctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgctgcttacctcccgagtc  
caagaccccgttaaccgccacctctcaaaatccggaaacacattccggcccagggtccacctgctgccgccgccgtcggaggag  
ctggccctgaacgagctggtgacgctgacgtgacctggcacgtggcttcagccccaaaggatgtgctggttcgtggctgcaggggtc  
acaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag  
40 catactgcgcgtggcagccgaggactggaagaagggggacaccttctctgcatggtggggccacgaggccctgccgctggccttc  
acacagaagaccatcgaccgcttggcgggtaaacccacctatgtcaatgtgtctgtgtcatggcggaggtggacggcacctgctac  
tgataatctaga

## +J Chain

agatctcaagaagatgaaaggattgtcttgttgacaacaaatgtaagtgtgccggattacttccaggatcatccgttctccgaagatc  
ctaatagaggacattgtggagagaaacatccgaattattgttctctgaacaacagggagaatatctctgatccacctcaccattgaga

accagatttgtgtaccatttgtctgacctcagctgtaaaaaatgtatcctacagaagtggagctggataatcagatagttactgctaccc  
agagcaatatctgtgatgaagacagtgtacagagacctgtacacttatgacagaaacaagtgtacacagctgtgggtccactcgt  
atatgggtggtgagacaaaatggtggaaacagccttaaccccagatgcctgctatcctgactaatctaga

5 Amino acid sequence:

2H7 scFv IgA WH WCH2 WCH3

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiyapsnlasgvparfsg  
sgsgtsysltisrveaadaatyccqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaelvrpgasvkmsckas  
gytftsynmhwwkqtrprglewigaiypngdtsynqfkkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy  
10 fdvwtgtttvtvssdqvpstpptpspstpspcchprlsrlhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgp  
pdrdlcgcysvssvlpgaepwnhgtftctaaypesktplatlksksgntfrpevhllpppseelalnelvtltclargfspkdvivr  
wlqgsqelprekyltwasrqepsqgtttfavtsilrvaedwkkgdtfscmvghelplafqtktidrlagkpthvnnsvvmaev  
dgtcy

15 +J Chain

rsqederivldnkckcaritsriirssedpnediverniriiiplnnrenisdptsplrtrfvyhlslsckkcdpteveldnqivtatq  
snicdedsatetcytydmkcytavvplvyggetkmvetaltpdacypd

20

## 78. 2H7 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcatgtttcagcttctgtaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagtacatgcactgg  
25 taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgtcgttcagtgga  
gtgggtctgggacctcttactcttcacaatcagcagagtgagggtgaagatgctgccactattactgccagcagtgagggttttaac  
ccaccacgttcggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggtgagtcggtgaggcctggggcctcagtgaaagatgctcgtgaaggcttctggctacacat  
ttaccagttacaatatgactgggtaaaagcagacacctagacagggcctggaatggattggagctatttatccaggaaatggtgatact  
30 tcctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctgcggtctatttctgtgcaagagtgggtgactatagtaacttactggtacttcgatgtctggggcacaggaccac  
ggtcaccgtctcttctgatcaggagcccaatcttctgacaaaactcacacatccccaccgtctcagcacctgaactcctgggggga  
ccgtcagttcttcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggtgacgtga  
gccacgaagacctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
35 agtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcacaaggcttctatccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacg  
40 cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiyapsnlasgvparfsg  
sgsgtsysltisrveaadaatyccqqsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
45 gytftsynmhwwkqtrprglewigaiypngdtsynqfkkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy  
fdvwtgtttvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgv

evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc  
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

5 **79. 2H7 scFv VH L11S (CSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggattttcaagtcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctctccagcaatcctgtctgcatctccaggggagaaggtcacaaatgacttgaggccagctcaagtgaagttacatgactgg  
taccagcagaagccaggatcctccccaaacctggatttatgccccatccaacctggcttctggagtcctgctcgcttcagtggca  
10 gtgggtctgggaccttactctctcacaaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgcttcggtgctgggaccaagctggagctgaaagatggcggtggctcggcggtggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggctgagtcggtgaggcctggggcctcagtgaaagatgctctgcaaggcttctggctacacat  
ttaccagttacaatatgactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggtgatact  
tctacaatcagaagttcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
15 atctgaagactctcggtctatttctgtgcaagagtggtgtactatagtaacttctactggtacttcgatgtctggggcacaggggaccac  
ggtcaccgtctcttctgatcaggagcccaaatctgtgacaaaactcacacatccccaccgtcctcagcacctgaactcctgggggga  
ccgtcagcttctcttccccccaaaaccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtga  
gccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgaggagc  
agtacaacagcagctaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctc  
20 tccaacaaagccctcccagccccatcgagaaaacaatctcaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacg  
cagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveadaatyccqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrprglewigaiypngdtsynqkfkkgkatltvdkssstaymqlssltedsavyfcarvvyysnsywy  
30 fdvwgtgtvtvssdqepkscdkthtspssapellggpsvlfppkpkdtlmisrtpetcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc  
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslsispkg

**78. G8-1 scFv VHL11S (SCS-S)H WCH2 WCH3**

Nucleotide sequence:

ggtgtaagcttgccgccatggatatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatg  
actcagctcagcctcccttatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgttacagttatttgctt  
ggatcagcagaaacagggaaaatctctcagctcctgtctcttttgcaaaaccttagcagaaggtgtgccatcaaggttcagtgg  
cagtggtatcaggcacacagtttcttgaagatcagcagcctgcagcctgaagattctggaagttatttctgcaacatcattccgataat  
40 ccgtggacgttcggtggaggcacccaactggagatcaaaggtggcggtggctcggcggtggtgggtcgggtggcgggcgatc  
gtcagcgggtccagctgcagcagcttgacctgagtcgaaaagcctggcgcttcagtgaagattctgcaaggtctctggtactca  
ttactggctacaatatgaactgggtgaagcagaataatgaaaagacgttgagtgattggaatattgatccttattatggtggtact  
acctacaaccggaagtcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac  
atctgaggactctgcagcttattactgtgcaagatcggtcggccctatggactactggggtaaggaaacctcagtcaccgtctcttctg  
45 atcaggagcccaaatcttctgacaaaactcacacatgccaccgtcctcagcacctgaactcctgggtggaccgtcagcttctcttctc  
ccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctg

aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt  
accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctccaacaagccctc  
ccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggtat  
gagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg  
5 ggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggac  
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
cctgtctccgggtaaatgatctagag

**Amino acid sequence:**

10 mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfssgsgs  
gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikggggsgggsgggssavqlqqsgpesekpgasvkiskcasgys  
ftgynmnwvkqnnkslewnidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
tsvtvssdqepkssdkthtccppsapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnakt  
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrldeltnqvsltlclvkgfyp  
15 sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhlehnhytqkslsispkg

**79.. G28-1 scFv VHL11S (CCS-P)H WCH2 WCH3**

**Nucleotide sequence:**

gttgtaagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatg  
20 actcagctcagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgttacagttatttgctt  
ggtatcagcagaaacagggaaaatctcctcagctcctgtctcttttgcacaaaccttagcagaaggtgtgccatcaaggttcagtg  
cagtggtatcagggcacacagttttctctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatcattccgataat  
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggatc  
gtcagcgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaagatttctgcaaggttctggttactca  
25 ttcactggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtggattgaaatattgatccttattatggtggtact  
acctacaaccggaagttcaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac  
atctgaggactctgcagcttattactgtcaagatcggtcggccctatggactactggggtcaaggaaacctcagtcaccgtctctctg  
atcaggagcccaaatctgtgacaaaactcacacatgtccaccgtccccagcactgaactcctgggtggaccgtcagttctctcttc  
ccccaaaacccaaggacacctcatgatctcccgaccctgaggtcatatgcgtgggtgggtggacgtgagccacgaagacctg  
30 aggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgt  
accgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaagccctc  
ccagcccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtat  
gagctgaccaagaaccaggtcagcctgacctgcctggctcaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg  
ggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagctcaccgtggac  
35 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
cctgtctccgggtaaatgatctaga

**Amino acid sequence:**

40 mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfssgsgs  
gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikggggsgggsgggssavqlqqsgpesekpgasvkiskcasgys  
ftgynmnwvkqnnkslewnidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
tsvtvssdqepkscdkthtccppsapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnakt  
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrldeltnqvsltlclvkgfyp  
sdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhlehnhytqkslsispkg  
45

**80. G28-1 scFv VH L11S (SCC-P)H WCH2 WCH3**

Nucleotide sequence:

gttgtaagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatg  
actcagctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgctt  
ggatcagcagaaacagggaaaatctcctcagctcctggtctcttttgcataaaccttagcagaaggtgtgccatcaagggtcagtg  
5 cagtgatcagggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtaacatcattccgataat  
ccgtggacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtggtgggtcgggtggcggcggatc  
gtcagcgggtccagctgcagcagctgtggacctgagtcggaaaagcctggcgcttcagtgaaagatttctgcaaggcttctggttactca  
ttcactggctacaatatgaactgggtgaagcagaataatgaaaagagccttgagtggattggaaatattgatccttattatggtgtact  
acctacaaccggaagttaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgac  
10 atctgaggactctgcatctattactgtgcaagatcggtcggccctatggactactgggtcaaggaaacctcagtcaccgtctcttctg  
atcaggagcccaaatcttctgacaaaactcacatgccaccgtgccagcacctgaactcctgggtggaccgtcagcttctctt  
cccccaaaacccaaggacaccctcatgatctcccgacccttgaggtcacatgcgtgggtgggtgacgtgagccacgaagaccct  
gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaagacaaagccgcgggaggagcagtacaacagcacg  
taccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatgcaaggagtacaagtgaaggtctccaacaaagccct  
15 cccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggga  
tgagctgaccaagaaccaggtcagcctgacctgctgtaaaggcttctatccaagcgacatcgccgtggagtgggagagcaatg  
ggcagccgggagaacaactacaagaccacgctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggac  
aagagcaggtggcagcaggggaacgtcttctcatgtccgtgatgatgaggctctgcacaaccactacacgcagaagagcctctc  
cctgtctccgggtaaatgatctaga

20

Amino acid sequence:

mvstaqlflglwltggrcdiqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs  
gtqfslkisslqpedsgsyfcqhhsdnptwtfgggtelkkgggsgggsgggssavqlqsgpeseekpgasvksckasgys  
ftgynmnwvkqnnkslewnidpyyggtynrkfkakatltvdkssstaymqlksltsedsavyycarsvgpmdywgqg  
25 tsvtvssdqepkssdkthtppcpapellggpsvflfppkpkdtlmisrtpetvvdvshedpevkfnwyvdgvevhnakt  
kpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsitclvkgyfp  
sdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

### 30 81. G28-1 scFv VH L11S mIgE CH2 CH3 CH4

Nucleotide sequence:

aagcttgccgcatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaatatgtttacagttatttgcttggat  
cagcagaaacagggaaaatctcctcagctcctggtctcttttgcataaaccttagcagaaggtgtgccatcaagggtcagtggcagt  
35 gatcagggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagttatttctgtaacatcattccgataatccgtg  
gacgttcggtggaggcaccgaactggagatcaaaggtggcgggtggctcgggcgggtggtgggtcgggtggcggcggatcgtcag  
cgggtccagctgcagcagctgtggacctgagtcggaaaagcctggcgcttcagtgaaagatttctgcaaggcttctggttactcattcact  
ggctacaatatgaactgggtgaagcagaataatgaaaagagccttgagtggattggaaatattgatccttattatggtgtactacctac  
aaccggaagttaagggaaggccacattgactgtagacaaatcctccagcacagcctacatgcagctcaagagtctgacatctga  
40 ggactctgcagctattactgtgcaagatcggtcggccctatggactactgggtcaaggaaacctcagtcaccgtctcttctgatcacg  
ttcgacctgtaacatcactgagccaccttgagctactccattcatcctgcgacccaatgcattccactccaccatccagctgtact  
gcttcattttatggccacatcctaaatgatgtctctgtcagctggctaattggacgatcgggagataactgatacattgcacaaactgttct  
aatcaaggagggaaggcaaaactagcctctacctgcagtaaatcaacatcactgagcagcaatggatgtctgaaagcaccttcacctg  
caaggtcacctcccaaggcgtagactatttggccacactcggagatgccagatcatgaccacgggggtgtgattacctacctgat  
45 cccaccagccccctggacctgtatcaaacgggtctcccaagcttacctgtctggtgggtggacctggaaagcgagaagaatgtcaa  
tgtgacgtggaaccaagagaagaagacitcagctcagcatccagtggtacactaagcaccacaataacgccacaactagtatcac

ctccatcctgcctgtagtgccaaggactggattgaaggctacggctatcagtgcatagtggaccaccctgattttccaagccattgt  
 gcgttccatcaccaagaccccaggccagcgtcagccccgaggtatatgtgtcccaccaccagaggaggagagcaggagcaa  
 acgcacactcacctgtttgatccagaacttctccctgaggatatctctgtgcagtggctgggggatggcaaactgatctcaaacagcc  
 agcacagtaccacaacaccctgaaatccaatggctccaatcaaggcttctcatcttcagtcgcttagaggtcgccaagacactctg  
 5 gacacagagaaaaacagttcacctgccaaagtatccatgaggcacttcagaaaccaggaaactggagaaaaacaatatccacaagc  
 ctggtgaacacctccctccgtccatcctagtaatatctagagg

Amino acid sequence:

mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs  
 10 gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikgggsgggsgggssavqlqsgpeseekpgasvkiskcasgys  
 ftgynmnwvkqnnkslewignidpyyggtynrkfkakatltvdksstaymqlksltsedsavyycarsvgpmdywgqg  
 tsvtvssdhvrpvniteptlellhsscdpnafhstiqlycfiyghilndvsvswlmdreitdtlaqtvlkeegklatscklniteqq  
 wmsestftckvtsqgvdyahtrrcpdheprgvitylippspldlyqngapkltclvdlseknvntwnqekktvsasqwy  
 tkhhnnattsitsilpvvakdwiegygyqcivdhpdpkpivrsitktpgqsapevyvfppeeesedkrtltcliqnffpedisv  
 15 qwlgdglisnshsttptlksnsgnqgfffsrlevaktlwtqrkqftcqihealqkprklektistslgnslrps

## 82. G28-1 scFv VH L11S mIgA WH WCH2 T4CH3

Nucleotide sequence:

20 aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgactca  
 gtctccagcctccctatctgcacatgtgggagagactgtcaccatcacatgtcgaacaagtgaataatgtttacagttatttgcttggtat  
 cagcagaaacagggaataatctcctcagctcctggctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagtg  
 gatcaggcacacagttttctgaagatcagcagcctgcagcctgaagattctggaagtatttctgcaacatattccgataatccgtg  
 gacgttcggtggagggcaccgaactggagatcaaaggtggcgggtggctcggcggtgggtgggtggcgggcggatcgtcag  
 25 cggctccagctgcagcagcttgacactgagtcggaaaagcctggcgttcagtgaagatttctgcaaggcttctggttactcattcact  
 ggctacaatatgaactgggtgaagcagaataatgaaagagccttgagtgattggaataattgatccttattatggtggtactacctac  
 aaccggaagttaagggaagggcaccattgactgtagacaaatctccagcacagcctacatgcagctcaagagtctgacatctga  
 ggactctgcagcttattactgtgcaagatcggcggcctatggactactgggtcaaggaacctcagtcaccgtctcttctgatcacat  
 ctgttctcctactactcctcctccaccttctgcccagcccagcctgtcactgcagcggccagctcttgaggacctgctcctgggttc  
 30 agatgccagcatcacatgtactctgaatggcctgagagatctgagggagctgtcttcacctgggagccctccactgggaaggatgc  
 agtgagaagaaagctgtgcagaattcctgcggctgctacagtggtccagcgtcctgctggtgtgctgagcgtggaacagtg  
 cgcatcattcaagtgcacagttaccatcctgagctgtgacacctaactggcacaattgccaaagtcacagtgaacacctccacccc  
 aggtccacctgctaccgccgccgtcgaggagctggcctgaatgagctcgtgtccctgacatgcctggtgcgagctttcaacccta  
 aagaagtgtggtgcgatggctgcatggaaatgaggagctgtcccagaaagctacctagtgttgagcccctaaggagccaggc  
 35 gagggagccaccacctggtgacaagcgtgtgctgtatcagctgaaatctggaacagggtgaccagtactcctgcatggtg  
 ggccacgaggcctgcccataacttcacccagaagaccatcgaccgtctgtcgggtaaacccaccaatgtcagcgtgtctgtgatc  
 atgcagagggagattgataatctagat

Amino acid sequence:

40 mvstaqlglillwltggrcdiqmtqspaslsasvgetvtitcrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsqsgs  
 gtqfslkisslqpedsgsyfcqhhsdnptwfgggteleikgggsgggsgggssavqlqsgpeseekpgasvkiskcasgys  
 ftgynmnwvkqnnkslewignidpyyggtynrkfkakatltvdksstaymqlksltsedsavyycarsvgpmdywgqg  
 tsvtvssdhicspptppppscqpslsqrpaledllgsdasitctlnlrdpegavftwepstgkdavqkkavqnsccgysvsv  
 lpgcaerwnsgasfkctvthpesdltgtiakvtvntfppqvhlpppseelanelvslclvrafnpkevlvrwlhgneelspey  
 45 lvfeplkepegattylvtsvlrvsaewkqgdqyscmvghealpmnftqtidrlsgkptnsvsvimsegd



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25  
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aagcttgccgccatggtatccacagctcagttccttgggttgctgctgctgtggcttacaggtggcagatgtgacatccagatgacta  
gtctccagcctccctatctgcatctgtgggagagactgtcaccatcacatgtcgaacaagtgaataatgtttacagttatttggcttggtat  
cagcagaaacagggaaaatctctcagctccttggtctcttttgcaaaaaccttagcagaaggtgtgccatcaaggttcagtggcagt  
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cgggtccagctgcagcagcttgacctgagtcggaaaagcctggcgcttcagtgaagattcctgcaaggcttctggttactattcact  
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tctgtctcagggacttcaccccgccaccgtgaagatcttacagtcgtctgcgacggcggcgggcacttcccccgaccatccag  
ctctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtggacttgcc  
accgctctaccacgcaggagggtgagctggcctccacacaaaagcagctcacccctcagccagaagcactggctgtcagaccgc  
acctacacctgccaggtcacctatcaaggtcacaccttgaggacagcaccaagaagtgtgcagattcaacccgagaggggtgag  
cgctacctaagccggcccagccgttcgacctgttcacccgaagtcgccacgatcacctgtctggtgggtggacctggcaccag  
caaggggaccgtgaacctgacctgggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaagcagcgca  
atggcacgttaaccgtcacgtccaccctgccggtgggcacccgagactggatcgagggggagacctaccagtgcaggggtgacc  
acccccacctgccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgtttgcgac  
gccggagtggccggggagccgggacaagcgcaccctgcctgcctgatccagaacttcagctgaggacatctcggtgcagt  
gtcgcacaacgaggtgcagctcccgacgccggcacagcacgacgcagccccgcaagaccaagggctccggcttctcgtctt  
cagccgcctggaggtgaccagggccgaatgggagcagaaagatgagttcatctgccgtgcagtcctgaggcagcgagccctc  
acagaccgtccagcgagcgggtgtctgtaaaccgggtaaatgataatctaga

mvstaqflgllllwtggrediqmtqspaslsasvgetvtitrtsenvysylawyqqkqgkspqllvsfaktlaegvpsrfsfgsgs  
gtqfslkisslqpedsyfcqhhsdnptwtfgggteleikggggsgggsgggssavqlqqsgpesekpgasvkiस्कasgys  
ftgynmnwvkqnnkgslewigndipyyggttnrkfkgkatltvdkssstaymqlksltsedsavyycarsvgpmdywggg  
tsvtvssdhvcsrdftppvtkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmvdldstasttqegelastqseltslk  
hwlsdrtytcqvtqyqghtfedstkkcadsnprgvsaylsrpspfdlfirksptitclvdlapskgtvnltsrasgkpvnhstrkee  
kqrngltvtstlpvgrdwiegetyqcrvthphlpralmrsttksgraaevyafatpewpgsrdrtrlaciqnfmpedisvq  
wlhnevqlpdarhsttqprktkgsgffvrsrlevtraeweqkdeficravheaaspsqtvravsvnpgk

40

45

atggtatccacagctcagttccttggggttgctgctgctgtggcttacagggtggcagatgtgacatccagatgactcagttccagcctcc  
ctatctgcatctgtgggagagactgtccatcacatgtcgaaacagtgaaaatgtttacagttattggcttggtatcagcagaaacag  
ggaaaaatcctcagctcctggctcttttgcaaaaaccttagcagaagggtgtgccatcaagggttcagttggcagttggtatcaggcacac  
agttttctctgaagatcagcagcctgcagcctgaagattctggaagttattctgtcaacatattccgataatccgtggacgttcgggtgg  
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agcagttctggacctgagtcggaaaagcctggcgcttcagtggaagatttctgcaagggttctgtgttactattcactggctacaatatg  
aactgggtgaagcagaataatgaaagagccttgagtgattggaaatattgatccttattatggtgggtactacctacaaccggaagtt  
caagggcaaggccacattgactgtagacaaatctccagcacagcctacatgcagctcaagagtctgacatctgaggactctgcagt  
ctattactgtgcaagatcggtcggccctatggactactggggtcaagggaacctcagtcaccgtctcttctgatcagccagttccctcaa



atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccaggt  
 ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaa  
 tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcgggtggctcgggcgggtgggtcgggtg  
 5 cggcggatcgtcagaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgaaagtttctgcaaggctc  
 tggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtgattggacagatttggcctgg  
 agatggtgataactacaatgaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact  
 cagcagcctagcatctgaggactctcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggact  
 actggggtcaaggaacctcagtcaccgtctcctctgatcag

10 Amino acid sequence:  
 metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdslwyqqipgppklliidasnlvsgipprf  
 sgsgsgtdftlnihpvekvdadyhcqqstedpwtfgggtkleikgggsgggsgggssqvqlqqsgaelvprgssvkisk  
 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavyfcarrettvgr  
 yyyamdywgqgtsvtvssdq

15 **88. HD37 VHL11S:**  
 Nucleotide sequence:

gggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaaagtttctgcaaggcttctggc  
 tatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtgattggacagatttggcctggagat  
 20 ggtgataactacaatgaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaactcagc  
 agcctagcatctgaggactctcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggactactg  
 ggtcaaggaacctcagtcaccgtctcctctgatcag

Amino acid sequence:  
 25 (gss)qvqlqqsgaesvprgssvkiskasgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadess  
 staymqlsslasedsavyfcarrettvgryyyamdywgqgtsvtvssdq

**89. HD37 scFv VHL11S:**  
 Nucleotide sequence:

30 Aagcttggcccatggagacagacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgctgacc  
 caatctccagcttcttggctgtgtcttagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagt  
 tatttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccag  
 ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaa  
 gtactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcgggtggctcgggcgggtgggtgggtcgggtg  
 35 gcggcgggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaaagtttctgcaaggct  
 tctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtgattggacagatttggcctg  
 gagatggtgataactacaatgaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaac  
 tcagcagcctagcatctgaggactctcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggac  
 tactggggtcaaggaacctcagtcaccgtctcctctgatcag

40 Amino acid sequence:  
 metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdslwyqqipgppklliidasnlvsgipprf  
 sgsgsgtdftlnihpvekvdadyhcqqstedpwtfgggtkleikgggsgggsgggssqvqlqqsgaesvprgssvkisk  
 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavyfcarrettvgr  
 45 yyyamdywgqgtsvtvssdq

**90. HD37 scFv IgAH hIgG1 WCH2 T4CH3**

Nucleotide sequence

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgtgaccc  
aatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagtt  
5 atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccaggt  
ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaaag  
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cggcgggagctcgcaggttcagctgcagcagctctggggctgagtcggtgaggcctgggtcctcagtgaaagattcctgcaaggctt  
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10 gagatggtgataactacaatggaaagtcaagggttaaagccactctgactgcagacgaatcctccagcacagcctacatgcaac  
tcagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggac  
tactggggtcaaggaacctcagtcaccgtctcctctgatcagccagtccctcaactccacctaccccatctccctcaactccacctac  
cccatctccctcatgctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcac  
gtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgcccctcaagtgggaagagcgctgttcaaggaccac  
15 ctgaccgtgacctctgtggctgtacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcactt  
gactgtgcctaccccgagtcgaagaccccgtaaccgccaccctctcaaaatccggaacacattccggcccagggtccacctg  
ctgccgccgctcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaggatgtgc  
tggttcgctgggtgcagggtcacaggagctgccccgcgagaagtacctgacttgggcatcccgccaggagcccagccaggggca  
ccaccacctcgtgtgaccagcactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtgggcca  
20 cgaggccctgccgtggccttcacacagaagaccatcgaccgcttggcgggttaaaccacccatgtcaatgtgtctgttgcattggc  
ggaggtggactgataatctaga

Amino acid sequence

metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdssylnwyyqqipgppklli ydasnlvsgipprf  
25 sgsrgstgdtflnihpvekvdadyhcqqstedpwtfgggtkleikgggsgggsgggssqvqlqqsgaesvrpgssvkisk  
asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadesstaymqlsslasedsavfyfcarrettvgr  
yyyamdywgqgtsvtvssdqpvspptpspstpspschprlsrlhrpaledlllgseailtctltglrdasgvtftwtpssgks  
avqgppdrldlcgysvssvlpgaepwnhgtftctaaypesktpltatlsksngtfrpevhllpppseelalnelvtlclargfsp  
kdvlvrwlqgsqelprekyltwasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghealplftqktidrlagkpthvnsv  
30 vmaevd

**91. HD37 scFv (SSS-S)H WCH2 WCH3**

Nucleotide sequence:

aagcttgccgccatggagacagacacactcctgctatgggtgctgctgctctgggtccaggctccactggtgacattgtgtgaccc  
35 aatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagtt  
atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcatccaatctagtttctgggatcccaccaggt  
ttagtggcagtggtctgggacagacttcacctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaaag  
tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcggtggctcgggcggtggtgggtcgggtgg  
cggcggatcgtcacaggttcagctgcagcagctctggggctgagctggtgaggcctgggtcctcagtgaaagattcctgcaaggcttc  
40 tggctatgattcagtagctactggatgaactgggtgaagcagaggcctggacagggtcttgagtggattggacagatttggcctgg  
agatggtgataactacaatggaaagtcaagggttaaagccactctgactgcagacgaatcctccagcacagcctacatgcaact  
cagcagcctagcatctgaggactctgcggtctatttctgtgcaagacgggagactacgacggtaggccgttattactatgctatggact  
actggggtcaaggaacctcagtcaccgtctcctctgatcaggagcccaaatctctgacaaaactcacacatccccaccgtcctcagc  
acctgaactcctgggtggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcaca  
45 tgcgtgggtgggtggacgtgagccacgaagacctgaggtcaagtgaactgttacgtggacggcgtggaggtgcataatgccaaaga  
caaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactgggtgaattggcaa

ggagtacaagtgaaggtctccaacaaagccctcccagcccccacgagaaaaccatctccaaagccaaagggcagccccgaga  
accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatc  
caagcgacatcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcctcccgtgctggactccgac  
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatgag  
5 gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdslwnwyqqipgppklliidasnlvsgipprf  
sgsgsgtdflnihpvekvdadyhcqqstedpwtfgggtkleikggggsgggsgggssqvlqqsgaelvrpgssvkisk  
10 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavfcarrettvgr  
yyyamdywgqgtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnw  
yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeltk  
nqvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqksls  
pgk

15

## 92. HD37 scFv VH L11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgccgcatggagacagacacactcctgctatgggtgctgctgcttgggtccaggtccactggtgacattgtgctgacct  
aatctccagcttcttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtgatagt  
20 atttgaactggtaccaacagattccaggacagccacccaaactcctcatctatgatgcacaaatctagtttctgggatcccaccaggt  
ttagtggcagtggtctgggacagacttaccctcaacatccatcctgtggagaaggtggatgctgcaacctatcactgtcagcaaa  
tactgaggatccgtggacgttcggtggaggcaccaagctggaaatcaaaggtggcgggtgctcgggcggtggtgggtcgggtgg  
cggcgaggagctgcaggttcagctgcagcagcttggggctgagtcggtgaggcctgggtcctcagtgaagattcctgcaaggctt  
ctggctatgcattcagtagctactggatgaactgggtgaagcagaggcctggacagggctttagtgattggacagatttggcctg  
25 gagatggtgataactacaatggaaagtcaagggtaaagccactctgactgcagacgaatcctccagcacagcctacatgcaac  
tcagcagcctagcatctgaggactctgcggctatttctgtcaagacgggagactacgacggtagggcgttattactatgctatggac  
tactgggtgcaaggaacctcagtcaccgtctcctctgacagagcccaaatcttctgacaaaactcacacatccccaccgtcctcag  
cacctgaactcctggggggaccgtcagcttctcttcccccaaaacccaaggacaccctcatgatctccggacccttgaggtca  
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaa  
30 gacaaagccgaggagagcagtagacaacagcagctaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatgg  
caaggagtacaagtcaaggtctccaacaaagccctcccagcccccacgagaaaacaatctccaaagccaaagggcagccccg  
agaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttct  
atcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccg  
acggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatg  
35 aggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

Amino acid sequence:

metdtlllwlwllwvpgstgdivltqspaslavslgqratisckasqsvdydgdslwnwyqqipgppklliidasnlvsgipprf  
sgsgsgtdflnihpvekvdadyhcqqstedpwtfgggtkleikggggsgggsgggssqvlqqsgaesvrpgssvkisk  
40 asgyafssywmnwvkqrpqglewigqiwpdgdtnyngkfkkgkatltadessstaymqlsslasedsavfcarrettvgr  
yyyamdywgqgtsvtvssdqepkssdkthtspssapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnw  
yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeltk  
nqvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqksls  
pgk

45

## 91. L6 VL

Nucleotide sequence:

atggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcccagctctccagc  
aatcctgtctgcatctccaggggagaagggtcacattgacttgaggccagctcaagtgttaagtttcatgaactggtaggcagcagaag  
ccaggatcctccccaaaccctggatttatgccacatccaatttggtctctgagttccctggcgttcagtggcgagtggtctgggacc  
5 tcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccactcacgttcgg  
tgctgggaccaagctggagctgaaagagctctctggtggcgggtggctcggcgggtgggtgggtggcggcgatct

Amino acid sequence:

10 Mdfqvqifsflisasvimrsgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiatsnlasefpgrfsge  
wsgtsylaisrveadaatyycqqwnsnpltfagtklelkelsgggsgggsgggsgggsg

## 92. L6 VH

Nucleotide sequence:

15 cagatccagttggtgcagcttgacactgagctgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtataccttca  
caaatatggaatgaactgggtgaagcaggctccaggaaagggttaagtggatgggctggataaacactacactggacagcca  
acatagctgatgactcaaggacgggttgccttctcttggaaacctctgcctacactgcctatttgagatcaacaacctcaaaatg  
aggacatggctacatattctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactctcacagctc  
cctctgatca

20 Amino acid sequence:

qiqlvqsgpelkpkpgetvkiskcasgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqi  
nnlknedmatyfcarsygnryadywgqgttlvtssd

## 93. L6 scFv

25 Nucleotide sequence:

atggattttcaagtgcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcccagctctccagc  
aatcctgtctgcatctccaggggagaagggtcacattgacttgaggccagctcaagtgttaagtttcatgaactggtaggcagcagaag  
ccaggatcctccccaaaccctggatttatgccacatccaatttggtctctgagttccctggcgttcagtggcgagtggtctgggacc  
tcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccactcacgttcgg  
30 tgctgggaccaagctggagctgaaagagctctctggtggcgggtggctcggcgggtgggtgggtggcggcgatctctgcag  
atccagttggtgcagcttgacactgagctgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtataccttcacaa  
actatggaatgaactgggtgaagcagggtccaggaaagggttaagtggatgggctggataaacactacactggacagccaaca  
tatgctgatgactcaaggacgggttgccttctcttggaaacctctgcctacactgcctatttgagatcaacaacctcaaaatgagg  
acatggctacatattctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactctcacagctcct  
35 ctgatca

Amino acid sequence:

40 mdfqvqifsflisasvimrsgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiatsnlasefpgrfsge  
wsgtsylaisrveadaatyycqqwnsnpltfagtklelkelsgggsgggsgggsgggslqiqlvqsgpelkpkpgetvkiskca  
sgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsygnry  
adywgqgttlvtssd

## 94. L6 VHL11S

Nucleotide sequence;

45 ctgcagatccagttggtgcagcttgacactgagtcgaagaagcctggagagacagtcagatctcctgcaaggcttctgggtatacct  
tcacaaactatggaatgaactgggtgaagcagggtccaggaaagggttaagtggatgggctggataaacactacactggacag

ccaacatatgctgatgacttcaaggacggtttgccttctctttggaacctctgcctacactgcctatttgcagatcaacaacctcaaaa  
atgaggacatggctacatatctgtgcaagatttagctatggttaactcacgttacgctgactactggggccaaggcaccactctcaca  
gtctcctctgatca

- 5 Amino acid sequence:  
qiqlvqsgpeskpkpgetvkiskasgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqi  
nnlknedmatyfcarfsygnrsryadywgqgttlvtssd

10 95. L6 VH L11S scFv

Nucleotide sequence;

Aagcttggtgttatggatttcaagtgagatttgcagcttctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctcc  
cagctctccagcaatctgtctgcattctccaggggagaagggtcacattgacttgcagggccagctcaagtgttaagttcatgaactggta  
ccagcagaagccaggatctctcccccacccctggatttatgccacatccaatttggcttctgagttccctggctcagtgaggcagtg  
15 ggtctgggacctcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccc  
actcacgttcgggtgctgggaccaagctggagctgaaagagctctctgggtggcgggtggctcgggcgggtgggtgggtcgggtggcggc  
ggatctctgcagatccagttggtgcagctctggacctgagtcgaagaagcctggagagacagtcagatctctgcaaggcttctggg  
tataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaaggggttaagtggtgggtgggataaacacctacact  
ggacagccaacatatgctgatgacttcaaggacggtttgccttctctttggaacctctgcctacactgcctatttgcagatcaacaac  
20 ctcaaaaatgaggacatggctacatatctgtgcaagatttagctatggttaactcacgttacgctgactactggggccaaggcaccac  
tctcacagctctctctgatca

Amino acid sequence:

- mdfqvqifsfllisasvmsrgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwiysnlasefpgrfsge  
25 wsgtsyslaisrveadaatyycqwnsnpltfagtklelkelsgggsgggsgggslqiqlvqsgpeskpkpgetvkiskac  
sgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarfsygnrsry  
adywgqgttlvtssd

30 96. L6 Or L6 VHL11S scFv IgAH hIgG1 WCH2 WCH3

Nucleotide sequence: (L6 VHL11S is shown)

aagcttggtgttatggatttcaagtgagatttgcagcttctgctaatacagtgcttcagtcataatgtccagaggacaaattgttctctccc  
agtctccagcaatctgtctgcattctccaggggagaagggtcacattgacttgcagggccagctcaagtgttaagttcatgaactggta  
35 cagcagaagccaggatctctcccccacccctggatttatgccacatccaatttggcttctgagttccctggctcagtgaggcagtg  
gtctgggacctcttactctctcgaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtggaatagtaaccca  
ctcacgttcgggtgctgggaccaagctggagctgaaagagctctctgggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcg  
gatctctgcagatccagttggtgcagctctggacctgagtcgaagaagcctggagagacagtcagatctctgcaaggcttctgggt  
ataccttcacaaactatggaatgaactgggtgaagcaggctccaggaaaggggttaagtggtgggtgggataaacacctacactg  
40 gacagccaacatatgctgatgacttcaaggacggtttgccttctctttggaacctctgcctacactgcctatttgcagatcaacaacct  
caaaaatgaggacatggctacatatctgtgcaagatttagctatggttaactcacgttacgctgactactggggccaaggcaccactc  
tcacagctctctctgatcagcagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgcgcacctg  
aactctgggggggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcg  
tggtgggtggacgtgagccacgaagacctgagggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaa  
45 gccgcggggaggagcagtaaacagcagctaccgtgtgggtcagcgtcctaccgtctgcaccaggactgggtgaatggcaaggga  
gtacaagtgcaaggtctccaacaaagccctccagccccatcgagaaaaacaatctcaaagccaaagggcagccccgagaacc

acaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtaaaaggcttctatccca  
gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggct  
ccttcttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctct  
gcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

5

Amino acid sequence:

mdfqvqifsfllisasvmsrgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwi yatsnlasefpgrfsge  
wsgtsylaisrveaadaatyycqqwnsnpltfagatkellelsgggsgggsgggslqqlvqsgpeskpkpgetvkiscka  
sgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsygnry  
adywgqgttlvtssdqpvstppstpspsscappellggpsvflfppkpkdtl misrtpevtcvvvdvshedpevkfnw  
yvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltk  
nqvsltclvkgyfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls  
pgk

10

15

97. L6 scFv VHL11S (SSS-S)H WCH2 WCH3

Nucleotide sequence:

aagcttgttgttatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggacaaattgttctctccc  
agtctccagcaatcctgtctgcattctccaggggagaaggtcacattgacttcgaggccagctcaagtgtaatgttcatgaactggtag  
cagcagaagccaggatcctccccaaaccctggatttatgccacatccaatttgcttctgagttccctggcgttcagtggcgagtg  
gtctgggacctcttactctctcgcaatcagcagagtgaggctgaagatgctgccacttattactgccagcagtggaatagtaacca  
ctcacgttcggtgctgggaccaagctggagctgaaagagctctctggtggcggtggtcgggcgggtggtgggtcgggtggcggcg  
gatctctgcagatccagttggtgcagcttgacgtgagcgaagaagcctggagagacagtaagatctcctgcaaggcttctgggt  
ataccttcacaaactatggaatgaactgggtgaagcaggtccaggaaaggggttaaagtggatgggctggataaacacctacactg  
gacagccaacatatgctgatgacttcaagggacggtttgcttctctttgaaacctctgcctacactgcctatttgcagatcaacaacct  
caaaaaatgaggacatggctacataattctgtgcaagatttagctatgtaactcacgttacgctgactactggggccaaggcaccactc  
tcacagtctcctctgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaactcctgggggggacc  
gtcagtcttctcttccccccaaaacccaaggacacctcatgatctccggacctgaggtcacatgcgtggtggtggacgtgag  
ccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca  
gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtct  
ccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgc  
ccccatcccggtgatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgacatcggcgtggag  
tgggagagcaatgggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaa  
gtcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc  
agaagagcctctccctgtctccgggtaaatgatctaga

35

Amino acid sequence:

mdfqvqifsfllisasvmsrgqivlsqspailsaspgekvtltcrasssvsfmnwyqqkpgsspkpwi yatsnlasefpgrfsge  
wsgtsylaisrveaadaatyycqqwnsnpltfagatkellelsgggsgggsgggslqqlvqsgpeskpkpgetvkiscka  
sgytftnygmnnwvkqapgkglkwmgwintytgqptyaddfkgrfafsletsaytaylqinnlknedmatyfcarsygnry  
adywgqgttlvtssdqepkssdkthtspssapellggpsvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwyvdgv  
evhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltc  
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispkg

40

45

98. P238 CH2

Nucleotide sequence:



accgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaa  
caatctccaaagccaaa

Amino acid sequence:

5 pellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw  
l  
ngkeykckvsnkalpapiektiskak

### 99. P238 CH2 WCH3

#### a. P238S CH2 WCH3

10 Nucleotide sequence:

cctgaactcctgggggatcgctcagttcttcttcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacat  
gcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac  
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaa  
ggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgaga  
15 accacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtgcaaaggcttctatc  
ccagcgacatcgccgtggagtgaggagcaatgggcagccggagacaactacaagaccacgcctcccgtgctggactccgac  
ggctccttctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgag  
gctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgatctaga

20

Amino acid sequence:

pellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdw  
l  
ngkeykckvsnkalpapiektiskakgqprepvytlppsrdeitknqvsitclvkgyfypsdiavewesngqpennyktpv  
l  
dsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgk

25

#### b. (SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

tgatcaggagcccaaatcttctgacaaaactcacatccccaccgtcctcagcacctgaactcctggggggatcgctcagttctct  
30 tcccccaaaacccaaggacaccctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccct  
gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg  
taccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccct  
cccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccggga  
tgagctgaccaagaaccaggtcagcctgacctgctgtgtaaaggcttctatcccagcgacatcgccgtggagtgaggagcaatg  
35 ggcagccggagacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggac  
aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
cctgtctccgggtaaatgatctaga

Amino acid sequence:

40 dqepkssdkhtspssapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeq  
ynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeitknqvsitclvkgyfypsdiavew  
esngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgk\*

### 45 100. a.CD16-6 low (ED) +NL+ (SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

ggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggatcgtcagttcttcttcccc  
 caaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtggagctgagccacgaagacctgaggt  
 caagttcaactggtacgtggacggcgtggaggtgcataatgccaaagccgcgggaggagcagtacaacagcacgtaccg  
 tgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtctccaacaaagccctccag  
 5 ccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggtgagc  
 tgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagcaatgggca  
 gccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttcttctctacagcaagctcaccgtggacaaga  
 gcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggctctgcacaaccactacacgcagaagagcctctccctgt  
 ctccgggtaaatgatctaga

Amino acid sequence:

mwqlllptallllvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg  
 eyrcqtnlstlspvqlvhighwlllqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsqsyf  
 crglvgsknvsssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkf  
 15 nwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdel  
 tknqvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqksls  
 lspgk

#### 101. b.CD16-6 low (ED)+HE4LP+(SSS-S)H P238S CH2 WCH3

Nucleotide sequence:

aagcttgccgccatgcctgctgtgccttaggccccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtctc  
 aggcaccgggtgcaatgaggactgaagatctccaaaggctgtggtgttctggagcctcaatggtacagggtgctcgagaaggaca  
 gtgtgactctgaagtgccaggagcctactcccctgaggacaattccacacagtggttcacaatgagagcctcatctcaagccagg  
 cctcgagctacttcattgacgtgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacctcagtgacccg  
 25 gtgcagctagaagtccatctcggtggtgtgtgtccaggccccctcgggtgggtgttcaaggaggaagaccctattcacctgaggtgtc  
 acagctggaagaacactgctctgcataaggtcacatattacagaatggcaaaggcaggaagtatttcatcataattctgacttctacat  
 tccaaaagccacactcaaagacagcggctcctactctgcagggggctgtgtggagtagaaaatgtgtcttcagagactgtgaacatc  
 accatcactcaaggtttggctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctgg  
 ggggatcgtcagttcttcttcccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtggtgg  
 30 acgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggg  
 aggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagt  
 caaggtctccaacaaagccctcccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgta  
 caccctgccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcg  
 ccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgctggtgactccgacggctccttcttct  
 35 ctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcagaggctctgcacaacc  
 actacacgcagaagagcctctccctgtctccgggtaaatgatctagaaa

Amino acid sequence:

mpacrlgplaaalllllfgftlvsgtgamrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassy  
 40 fidaatvddsgeyrcqtnlstlspvqlvhighwlllqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi  
 pkatlkdsqsyfcrglvgsknvsssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmsrtpevtcvvv  
 dvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgppre  
 pqvytlppsrdeltnqvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmh  
 ealnhnhtqkslsispgk

**102. CD16-9 high (ED) (SSS-S)H P238S CH2 CH3**

**a. CD16-9 high (ED)NL+(SSS-S)H P238S CH2 CH3**

Nucleotide sequence:

5 gttgtaagcttgccgcatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgaggactgaagatctccca  
aaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggacagtgtgactctgaagtgccagggagcctactcccct  
gaggacaattccacacagtgtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacg  
acagtggagagtacaggtgccagacaaacctctccacctcagtgacccgggtgcagctagaagtccatcggctggctgttgctcc  
aggccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacat  
10 attacagaatggcaaaggcaggaagtatttcatcataattctgacttctacattccaaaagccacactcaagacagcggctcctactt  
ctgcaggggggcttttgggagtaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcaggagcccaaat  
cttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggggggatcgtcagttcttcttccccccaaaacccaa  
ggacacctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttaact  
ggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagc  
gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcga  
15 gaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa  
ccaggtcagcctgacctgctgtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac  
aactacaagaccacgctcccgtgtgactccgacggctccttcttctctacagcaagctcaccgtggacaagagcaggtggca  
gcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa  
atgatctagaaa

20

Amino acid sequence:

mwqlllptallllvsagmrtdlpkavvflepqwyrlekdsvtlkcgayspednstqwfhnslissqassyfidaatvddsg  
eyrcqtnlstldpvlqlevhigwllqaprwwfkeedpihlrchswkntalhkvtlylqngkgrkyfhnsdfyipkatlkdsqsyf  
crglfgsknvsssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmisrtpvctvvvdvshedpevkfn  
25 wyvdgvevhnatkpreeqnystyrvsvltvlhqdwlngkeykckvsnkalpapietkiskakgqprepvytlppsrdeit  
knqvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdkswqqgnvfscsvmhleahnhytqksls  
spgk

**CD16-9 high (ED)+HE4LP+hIgG1 (SSS-S)H P238S CH2 CH3**

30 Nucleotide sequence:

aagcttgccgcatgctgtgctgcttaggcccgttagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtctc  
aggcaccgggtgcaatgaggactgaagatctcccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggaca  
gtgtgactctgaagtgccagggagcctactcccctgaggacaattccacacagtgtgttcacaatgagagcctcatctcaagccagg  
cctcgagctacttcattgacgctgccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacctcagtgacccg  
35 gtgcagctagaagtccatcggctggctgtgtcctcaggccccctgggtgggtgttcaaggaggaagaccctattcacctgaggtgc  
acagctggaagaacactgctctgcataagggtcacatatttacagaatggcaaaggcaggaagtatttcatcataattctgacttctacat  
tccaaaagccacactcaaagacagcggctcctacttctgcagggggcttttgggagtaaaaatgtgtcttcagagactgtgaacatca  
ccatcactcaaggtttggctgatcaggagcccaaatcttctgacaaaactcacacatccccaccgtcctcagcacctgaactcctggg  
gggatcgtcagttcttcttccccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtgga  
40 cgtgagccacgaagaccctgaggtcaagttaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcggga  
ggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgc  
aaggtctccaacaaagccctccagccccatcgagaaaacaatctccaaagccaaagggcagccccgagaaccacaggtgtac  
acctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgctgtaaggcttctatccagcgacatcgc  
cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtgactccgacggctccttctcctct  
45 acagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca  
ctacacgcagaagagcctctcctgtctccgggttaaatgatctagaaa

Amino acid sequence:

mpacrlgplaaallslfllvsgtgamrtedlpkavvflepqwyrlekdsvtlkcqgayspednstqwfhnslissqassy  
fidaatvddsgeyrcqtnlstlspvqlvhighwillqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi  
5 pkatlkdsgsyfcrglvgsknvssetvnititqgladqepkssdkthtspssapellggssvflfppkpkdtlmisrtpvctcvvd  
vshedpevkfnwyvdgvevhnaktprreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprep  
qvylppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhe  
alhnhytqkslslspgk

10

**103. a. CD16 ED low (native leader)**

Nucleotide sequence:

aagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatgcccagctgaagatctcccaaaggc  
tgtggtgttctggagcctcaatggtacaggggtgctcgagaaggacagtgtagctctgaagtgccaggagcctactcccctgagga  
15 caattccacacagtggtttcacaatgagagcctcatctcaagccaggcctcgagctacttcattgacgctgccacagtcgacgacagt  
ggagagtacaggtgccagacaaacctctccacctcagtgacccgggtgcagctagaagtccatcgcgctggctgttgctccaggc  
ccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacatatta  
cagaatggcaaaggcaggaagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcggctcctacttctgc  
aggggggctgttgggagtaaaaatgtgtcttcagagactgtgaacatcacatcactcaagggttggtgatcaaaa

20

Amino acid sequence:

mwqlllptalllvsagmrtedlpkavvflepqwyrlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg  
eyrcqtnlstlspvqlvhighwillqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsgsyf  
25 crglvgsknvssetvnititqgladq

25

**b. CD16 ED low (HE4 leader)**

Nucleotide sequence

aagcttgccgccatgcctgctgtgccttagggcccgctagccgccgccctcctcctcagcctgctgctgttcggcttcaccctagtctc  
aggcaccgggtgcaatgcccagctgaagatctcccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggaca  
30 gtgtgactctgaagtgccaggagcctactcccctgaggacaattccacacagtggtttcacaatgagagcctcatctcaagccagg  
cctcgagctacttcattgacgctgccacagtcgacgacagtgagagtagagtgccagacaaacctctccacctcagtgacccg  
gtgcagctagaagtccatcgcgctggctgttgctccaggccctcgggtgggtgttcaaggaggaagaccctattcacctgaggtgtc  
acagctggaagaacactgctctgcataaggtcacatatttacagaatggcaaaggcaggaagtattttcatcataattctgacttctacat  
tccaaaagccacactcaaagacagcggctcctacttctgcaggggggctgttgggagtaaaaatgtgtcttcagagactgtgaacatc  
35 accatcactcaagggttggtgatcaaaa

Amino acid sequence

mpacrlgplaaallslfllvsgtgamrtedlpkavvflepqwyrlekdsvtlkcqgayspednstqwfhnslissqassy  
40 fidaatvddsgeyrcqtnlstlspvqlvhighwillqaprvwfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyi  
pkatlkdsgsyfcrglvgsknvssetvnititqgladq

**104. a. CD16 ED high (native leader)**

Nucleotide sequence:

45

gttgtaagcttgcgccaatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatcgggactgaagatctccca  
aaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggacagtgtagctctgaagtgcaggaggcctactccct  
gaggacaattccacacagtgtttcacaatgagagcctcatctcaagccaggcctcagctacttcattgacgtgccacagtcgacg  
acagtggagagtacaggtgccagacaaacctctccacctcagtgacccgggtgcagctagaagtccatacggctggctgttgctcc  
5 agggccctcgggtgggtgttcaaggaggaagacctattcacctgaggtgtcacagctggaagaacactgctctgcataaggtcacat  
atctacagaatggcaaaggcaggaagtatttcatcataattctgacttctacattccaaaaggccacactcaaagacagcggctcctactt  
ctgcagggggcgttttgggagtaaaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggctgatcaaa

Amino acid sequence:

10 mwqlllptallllvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg  
eyrcqtnlstlspvqlvhighwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhhsndfyipkatlkdsqsyf  
crglfgsknvssetvnititqgladq

#### b. CD16 ED high (HE4 leader)

15 Nucleotide sequence:

aagcttgcgccaatgcctgctgtgcctaggcccgtagccgccgcccctcctcagcctgctgctgttcggcttcaccctagtctc  
aggcaccgggtgcaatcgggactgaagatctcccaaaggctgtggtgttctggagcctcaatggtacaggggtgctcgagaaggaca  
gtgtgactctgaagtccaggaggcctactcccctgaggacaattccacacagtgtttcacaatgagagcctcatctcaagccagg  
cctcagctacttcattgacgtgccacagtcgacgacagtgagagtagcaggtgccagacaaacctctccacctcagtgacccg  
20 gtgcagctagaagtccatacggctggctgtgtcctcaggccctcgggtgggtgttcaaggaggaagacctattcacctgaggtgtc  
acagctggaagaacactgctctgcataaggtcacataattacagaatggcaaaggcaggaagtatttcatcataattctgacttctacat  
tccaaaaggccacactcaaagacagcggctcctacttctgcagggggcgttttgggagtaaaaatgtgtcttcagagactgtgaacatca  
ccatcactcaaggtttggctgatcaaa

25 Amino acid sequence:

mpacrllgplaaalllllfgftlvsgtgamrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassy  
fidaatvddsgeyrcqtnlstlspvqlvhighwlllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhhsndfyi  
pkatlkdsqsyfcrglfgsknvssetvnititqgladq

30

#### 105. 2e12 scFv (SSS-S)H P238S CH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagttta  
35 atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctgggtccctgccagg  
tttagtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaat  
aggaaggttcttgacgttcgggtggagggaccaagctggaaatcaaacgggtggcggtggctcgggcggaggtgggtcgggt  
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctgggtggcggcctcacagagcctgtccatcacatgcaccgtct  
caggggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
40 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggaactccaagagccaagtttcttaaaaatgaa  
cagctctgaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
tggggggatcgtcagcttctccttccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggt  
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
45 ggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag  
tgcaaggtctccaacaaagccctccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgt

acaccctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
 cactacacgcagaagagcctctccctgtctccgggtaaaaggatccttcgaacctgctcccatcctggccattacctaatactcagt  
 5 aaatggaattttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaaggga  
 aagtgtacgccctgtataaatcgat

Amino acid sequence:

mdfqvqifsflisasvmsrgvdivltqspaslavslgqratiscrasesveyvtslmqwyqqkpgqppkllisaasnvesgvp  
 10 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgggsgqvqlkesgpglvapsqslsit  
 ctvsgfsltygvnwvrqppgkglewlgmiwgdgstdynsalksrlsitkdnksqvflkmnslqtdtdaryycardgysnfh  
 yyvmdywgqgtsvtvssdlepkssdkthtspapellggssvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwy  
 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeitkn  
 qvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp  
 15 gkadpsnllpswaitlisvngifviccltycfaprerrrrmerlrresvrpv

#### 106. 10A8 scFv (SSS-S)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacatccagatgacac  
 20 agtctccatcctcactgtctgcatctctgggaggcaaaagtcaccatcacttgcaaggcaagccaagacattaagaagtatataggttg  
 taccaacacaagcctggaaaagggtcccagggtgctcatatattacacatctacattacagccaggcatcccatcaaggttcagtggaa  
 gtgggtctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactattattgtcaacagtatgataatcttcc  
 attgacgttcggctcggggacaaagtggaaataaaacgggtggcgggtggctcgggcgggtgggtgggtcgggtggcgccggatct  
 gatgtacagcttcaggagtcaggacctggcctcgtgaaaccttctcagtcctctgtctcactgctctgtcactggctactccatcacc  
 25 agtggtttctactggaactggatccgacagtttccgggaacaaactggaatggatgggccacataagccacgacggtaggaataac  
 tacaacctatctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtgactactgagga  
 cacagctacatatttctgtgcaagacactacgtagtagcggagctatggactactggggtcaaggaacctcagtcaccgtctcctct  
 gatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagttctctctt  
 cccccaaaacccaaggacacctcatgatctcccggaccctgaggtcacatgcgtgggtgggtgacgtgagccacgaagacct  
 30 gaggtcaagttcaactgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacg  
 taccgtgtgtgcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgcaggctccaacaaagccct  
 ccagcccccatcgaaaaacatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccggga  
 tgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg  
 ggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctctctctacagcaagctcaccgtggac  
 35 aagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc  
 cctgtctccgggtaaaagcggatccttgaacctgctccatcctgggccattacctaatactcagtaaatggaattttgtgatatgctgc  
 ctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcga  
 t

40 Amino acid sequence:

mdfqvqifsflisasvmsrgvdiqmtqspsslsaslggkvititckasqdikkyigwyqhkpkgprlliiyytstlqpgipsrfs  
 sgsgrdyslsimlepediatyycqydnpltfsgstkleikrgggsgggsgggsgggsgdvqlqesgpglvkpsqslstcvtgys  
 itsgyfwnwirqfpgnklemwghishdgrnynpslinrisitrdtsknqffklssvttdtatyfcarhygssgamdywgqgt  
 svtvssdlepkssdkthtspapellggssvflfppkpkdtlmsirtpevtcvvvdvshedpevkfnwyvdgvevhnaktk  
 45 preeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeitknqvsltlvkgfyps

diavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgkadpsnllpswa  
itlisvngifviccltycfaprcrerrrnerlrresvrpv

5    **107.    40.2.36 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT**

Nucleotide sequence:

Amino acid sequence:

10

108.    2H7 scFv VHL11S (SSS-P)H P238SCH2CH3-hCD80TM/CT

Nucleotide sequence:

15

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacatgacttgaggccagctcaagtgttaattacatgcactgg  
taccagcagaagccaggtatctccccaaacctggatttatgccccatccaacctggcttctggagctccctgctcgttcagtgga  
gtgggtctgggaccttactctctcacatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtctgggaccaagctggagctgaaagatggcgggtggctcggcggtggatctggaggaggtgggagc  
tctcaggcttatctacagcagctctggggtgagtcggtagggcctggggcctcagtgaaagatgctcgaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggatggagctatttatccaggaaatggtgatact  
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
atctgaagactctcggtctatttctgtgcaagagtgggtactatagtaacttactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatctggagcccaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggggga  
tcgtcagttctcttcccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgggtgacgtga  
gccacgaagacctgaggtcaagtcaactgtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc  
agtacaacagcagctaccgtgtgtgcagcgtctcaccgtctgcaccaggactgggtgaatggcaaggagtacaagtgaaggctc  
tccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtacacctg  
ccccatccgggatgagctgaccaagaaccaggtcagctgacctgctgctgtaaaggcttctatcccagcgacatcgccgtgga  
gtgggagagcaatgggcagccggagaacaactacaagaccacgctcccgctgctggactccgacggctccttctctctacagca  
agctcaccgtggacaagagcaggtggcagcaggggaacgtcttctatgctcctgtatgcagaggtctgcacaaccactacacg  
cagaagagcctctccctgtctccgggtaaagcggatccttgaacctgctccatcctgggccattaccttaattcagtaaatggaatt  
tttgtgatatgctgctgacctactgcttggcccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgc  
cctgtataaatcgat

35

Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypslnlasgvparfsg  
sgsgtsysltisrveaadaatyycqqwsfnpptfgagtklelkdggsgggsgggssqaylqqsgaesvrpgasvkmsckas  
gytftsynmhwwkqtrqglewigaiypngdtsynqkfkgkatltvdksstaymqsltsedsavfcarvvysnsywy  
fdvwgtgtvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgve  
vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltknqvsitcl  
vkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslspsgkadps  
nllpswaitlisvngifviccltycfaprcrerrrnerlrresvrpv

45

109.    G19-4 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac  
agactacatctccctgtctgcctctctgggagacagagtcaccatcagttgcagggaagtcaggacattcgcaattattttaaactgg

tatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtgccatcaagggttcagtggcag  
 tgggtctggaacagattattctcaccattgccaacctgcaaccagaagatattgccacttacttttccaacagggttaatacgttccg  
 tggacgttcggaggagcaccaaactggaaccaacgggagctcgggtggcgggtggctcgggcgggtgggtgggtcgggtggcg  
 cggatctatcgatgaggtccagctgcaacagcttggaacctgaactggagaacctggagcttcaatgtcctgcaaggcctctggttac  
 5 tcattcactggctacatcgtaactggctgaagcagagccatggaaagaaccttgagtggattggacttattaatccatacaaaaggtctt  
 actacctacaaccagaaattcaagggaaggccacattaactgtagacaagtcacccagcacagcctacatggagctcctcagctctg  
 acatctgaagactctgcagctctattactgtgcaagatctgggtactatgggtgactcggactggacttcgatgtctggggcgcaggagac  
 cacggtcaccgtctcctctgatctggagcccaaatctctgacaaaactcacacaagcccaccgagcccagcacctgaactcctggg  
 gggatcgtcagctctcctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgggga  
 10 cgtgagccacgaagacctgaggtcaagttcaactggtagctggagcggcgtggaggtgcataatgccaagacaaagccgcggga  
 ggagcagtacaacagcacgtaccgtgtgggtcagcgtcctcaccgtctgcaccaggactgggtgaatggcaaggagtacaagtgc  
 aaggtctccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtac  
 acctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcgaaggcttctatcccagcgacatcgc  
 cgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcctct  
 15 acagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacca  
 ctacacgcagaagagcctctcctgtctccgggtaagcggatccttgaacctgctccatcctgggccattaccttaatctcagtaa  
 atggaattttgtgatatgctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaaa  
 gtgtacgcctgtataaatcgatactcgag

20 Amino acid sequence:  
 mdfqvqifsfllisasvimsgvdiqmtqtsslsaslgdrvtiscrasqdirnylnwyqqkpdgtvkllyytsrlhsgvpsrfs  
 gsgtdysltianlqpediatyfcqqntlpwtfgggtklvtkrelggsgsgsgsgsgsgsidevqlqsgpelvkgasmsckas  
 gysftgyivnwlkqshgknlewiglinpykgltytnqkfkkgkatltvdkssstaymellsitsedsavyycarsgyygdswyf  
 dvwgagttvtvssdlepkssdkthtspspapellgssvflfpkpkdtlmsrtpetvctvvvdvshedpevkfnwyvdgve  
 25 vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdektknqvsitcl  
 vkgfypsdiavewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslspgkadps  
 nllpswaitlisvngifviccltycfaprerrrrmerlrresvrpv

### 30 110. 2e12 scFv (SSS-P)H WCH2 WCH3-hCD80TM/CT

Nucleotide sequence:  
 aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc  
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
 atgcagtggtagcaaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccagg  
 35 tttagtggcagtggtgtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
 aggaaggttccttgacgttcgggtggagggaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt  
 ggcggcggatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgcctcacagagcctgtccatcacatgcacctgtc  
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatagggtg  
 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagtttcttaaaaatgaa  
 40 cagctctgcaaaactgatgacacagccagataactgtgccagagatggtatagtaactttcattactatgttatggactactgggtcaa  
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatcccaccgtccccagcacctgaactcc  
 tggggggaccgtcagcttctccttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtgggtgg  
 tggacgtgagccacgaagacctgaggtcaagttcaactggtagctggacggcgtggaggtgcataatgccaagacaaagccgcg  
 ggaggagcagtacaacagcacgtaccgtgtgtgcagcgtcctcaccgtctgcaccaggactgggtgaatggcaaggagtacaag  
 45 tgcaaggtctccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggcgaaggcttctatcccagcgacatc



gccgtggagtgaggagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctccctgtctccgggtaagcggatccttcgaacctgctcccatcctgggccattacctaatactcagt  
aatggaattttgtgatatgctgcctgacactactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaaggga  
5 aagtgtacgccctgtataaatcgat

Amino acid sequence

mdfqvqifsfllisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfsghsgstgdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit  
10 ctvsgfsltygvnwvrpppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqddtaryycardgysnfh  
yyvmdywgqgtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeitkn  
qvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp  
gkadpsnllpswaitlisvngifviccltycfaprerrrrerlrresvrpv

15

# 111. 2e12 scFv IgA WH WCH2 T4 CH3-hCD80TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
20 atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtccctgccagg  
tttagtggcagtgggctctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
aggaaggttccttgacgttcgggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt  
ggcggcgatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgcctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
25 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttttataaaatgaa  
cagctctgaaactgatgacacagccagataactactgtgccagagatggtatagtaactttcattactatgttatggactactgggtgcaa  
ggaacctcagtcaccgtctcctcagatcagccagttccctcaactccacctacccatctcctcaactccacctacccatctcctca  
tgctgccacccccgactgtcactgcaccgaccggcctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgacc  
ggcctgagagatgcctcaggtgtcaccttcacctggacgcctcaagtgggaagagcgtgttcaaggaccacctgacctgacct  
30 ctgtggctgtacagcgtgtccagtgctcctgccgggctgtgccgagccatggaacctgggaagacctcactgtcactgtgcctac  
cccagatccaagaccccgtaccgccacctctcaaaatccggaaacacattccggcccagggtccacctgtgccgccgccgt  
cggaggagctggcctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagcccaaggatgtgctggttcgctggt  
gcaggggtcacaggagctgccccgcgagaagtacgtgacttgggcatccggcaggagcccagccagggcaccaccacctcg  
ctgtgaccagcactatgcgcgtggcagccgaggactggaagaagggggacacctctcctgcattggtggccacgaggccctgc  
35 cgctggccttcacacagaagaccatcgaccgcttggcgggtaaacccacctatgcaatgtgtctgttgcattggcggaggtggacg  
cggatccttcgaacaacctgctccatcctgggccattaccttaatctcagtaaatggaattttgtgatatgctgcctgacctactgctt  
gccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgatac

Amino acid sequence:

mdfqvqifsfllisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfsghsgstgdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit  
ctvsgfsltygvnwvrpppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqddtaryycardgysnfh  
yyvmdywgqgtsvtvssdqpvpstptpsptpspschprlsllhrpaledlllgseailtctltglrdasgvftwtpssgksa  
vqppdrdlcgcysvssvlpgaepwnhgtftctaaypesktpitltsksgntfrpevhllpppseelalnelvtlclargfspk  
45 dvlvrwlqsgqlprekyltwasrqpsqgtttfavtsilrvaaedwkkgtfscmvghlealplafktidrlagkpthvnsvv  
maevdadpsnllpswaitlisvngifviccltycfaprerrrrerlrresvrpv

### 112. 2e12scFv IgE WCH2WCH3WCH4-hCD80TM/CT

Nucleotide sequence:

5 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaaagtta  
atgcagtggtagcaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtcctgccagg  
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
aggaaggttcttgacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcggggcggaggtgggtcgggt  
ggcggcggatctcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct  
10 cagggttctcattaaccggctatggtgtaaactgggtcggcagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagttttctaaaaatgaa  
cagctctgaaaactgatgacacagccagataactactgtgccagagatggtatagtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctcctcagatcacgtctgtccagggaactcaccgccaccgtgaagatcttacagtcgtcctgcgacg  
gcggcgggacacttcccccgacctccagctcctgtgcctcgtctctgggtacacccagggactatcaacatcacctggctggag  
15 gacgggcaggtcatggacgtggacttgcaccgcctctaccacgcaggagggtgagctggcctccacacaaagcgagctcaccc  
tcagccagaagcactggctgtcagaccgcacctacacctgccaggtcacctatcaaggtcacacctttgaggacagcaccaagaag  
tgtgcagattccaacccgagaggggtgagcgctacctaagccggccagcccgttcgacctgttcatccgcaagtcgccacgat  
cacctgtctggtggtggacctggcaccagcaaggggaccgtgaacctgacctggtcccgggccagtggaagcctgtgaaccac  
tccaccagaaaggaggagaagcagcgcaatggcacgttaaccgtcacgtccaccctgccggtgggacccgagactggatcgag  
20 ggggagacctaccagtgcagggtgacccacccccacctgccaggggcctcatgcggtccacgaccaagaccagcggcccgcg  
tgctgccccggaagtctatgcgtttgcgacggcgagtgccggggagccgggacaagcgaccctcgctgcctgatccagaac  
ttcatgcctgaggacatctcgggtgcagtggtgcacaacgaggtgcagctccggacgcccggcacagcacgacgcagccccgc  
aagaccaaggggtccggcttcttcgtctcagccgctggaggtgaccagggccgaatgggagcagaaaagatgagttcatctgccg  
tgagtcctatgaggcagcgagccccctacagaccgtccagcgagcggtgtctgtaaatcccggtaaagcgatccttcgaagctcc  
25 catcctggggcattaccttaatctcagtaaatggaattttgtgatatgtgcctgacctactgcttggcccaagatgcagagagagaag  
gaggaatgagagattgagaagggaagtgtacgccctgtataaatcgata

Amino acid sequence:

30 mdfqvqifsflisasvmsrgdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgqvqlkesgpglvapsqslsit  
ctvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh  
yyvmdywgqgtsvtvssdhvcsrdftpptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmvdvlstattqeg  
elastqseltsqkhwlslrtytcqvyqghtfedstkkcadsnprgvsaylsrspfdlfrksptitclvdlapskgtvnltwsras  
gkpvnhstrkeekqrmgtltvtstlpvgrdwiegetyqervthphlpralmrsttktsgpaaapevyafatpewpgsrdrkrtlacli  
35 qnfmpedisvqwlhnevqlpdarhsttqprktkgsgffvfrlevtraeweqkdeficravheaaspsqtvravsvnpgkadb  
sklpswaitlisvngifviccltycfaprerrrrerlrrsvrpv

### 113. mFADD-TM/CT

MFADD scscFv

40 Nucleotide sequence:

ggttgatgactctgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctca  
agttcttgtgccgcgagcgcgtgagcaaacgaaagtggagcgcgtgcagagtgccctggacctgttcacggtgctgctggagca  
gaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctgga  
cgacttcgaggcggggacggcgaccgctgcggcccgggggaggcagatctgcaggtggcatttgacattgtgtgacaatgtg  
45 gggagagactggaaaagactggcccgcgagctgaaggtgtctgaggccaagatggatgggattgaggagaagtacccccgaagt  
ctgagtgagcgggtaaggagagagctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactggtcaaggcg

ctgcggacctgcaggctgaatctgggtgacctgggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtccccagt  
actaagggttcaactgtgtcttctcagaacacctgactcagatcgat

Amino acid sequence:

5 Vvdpsnmdpflvllhslsgslsgndlmelkflcrervskrklerqvsgldlftvlleqndlerghtgllrellaslrhdllqrlddfea  
gtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglikalrtc  
rlnlvadlveeaquesvksenmspvldstvsstetp

10 114. 2e12 scFV (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaggtgtgaatattatgcacaagtta  
atgcagtggtagcaacagaaaccaggacagccacccaaactctcatctgtgcatccaacgtagaatctgggggtccctgccagg  
15 tttagtggcagtggtgtgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgcagcaagt  
aggaaggttcttgacgttcggtggagggcaccaagctggaaatcaaacggggtggcgggtggctcgggaggagtggtcgggt  
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctgggtggcgcctcacagagcctgtccatcacatgcacctgt  
cagggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaatgaa  
20 cagctctgcaaactgatgacacagccagataactgtgccagagatggttatgtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
tgggtggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccggaccttgaggtcacatgcgtggtggt  
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
ggaggagcagtagaacagcacgtacctgtggtcagcgtcctaccgtctgcaccaggactggctgaatggcaaggagtacaag  
25 tgcaaggctctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt  
acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctcctgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggaccattcctggtgctgctgcactcgtgt  
30 ccggcagcctgtcgggcaacgatctgatggagctcaagttcttggccgcgagcgcgtgagcaaacgaaagctggagcgcgtgca  
gagtggtcgtggacctgttcacggtgctgctggagcagaacgacctggagcgcgggcacaccgggctgctgcgcgagtgctggc  
ctcgtgctgcggcagacgatctactgcagcgcctggacgacttcgagggcggggacggcgaccgctgcggccgggggagggag  
atctgcaggtggcatttgacattgtgtgtgacaatgtggggagagactggaaaagactggccgcgagctgaagggtgtctgaggcc  
aagatggatgggattgaggagaagtacccccgaagtctgagtgagcgggtaaggagagctgaaagtctggaagaatgctgaga  
35 agaagaacgcctcgggtggccggactggtcaaggcgtgcggacctgcaggctgaatctggtgggtgacctggtggaagaagccc  
aggaatctgtgagcaagagtgagaatatgtccccagtactaagggttcaactgtgtcttctcagaacacctgactcagatcgat

Amino acid sequence:

mdfqvqifsfllisasvimsvrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaansvesgvp  
40 arfsgsgsgtdfslnhpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit  
ctvsgfsltgygvnwvrppgkglewlgmiwgdstdynsalksrsltkdnsksqvflkmnslqddtaryycardgysnfh  
yyvmdywgqgtsvtvssdlepkssdkthtspapellggssvflfppkpkdtlmisrtpetvcvvvdvshedpevkfnwy  
vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeyckvsnkalpapiektiskakgqprepvytlppsrdeltn  
qvsitclvkgyfypsdiavewesngqpennkytppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp  
45 gkadpsnmdpflvllhslsgslsgndlmelkflcrervskrklerqvsgldlftvlleqndlerghtgllrellaslrhdllqrlddfea

gtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglivkalrtc  
rlnlvadlveeaquesvksenmspvldstvsstetp

**115. 2e12 scFv (SSS-P)H WCH2WCH3-mFADD-TM/CT**

5 Nucleotide sequence:

aagcttatggatttcaagtgcagatttgcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctgcagagccagtgaaagtgtgaatattatgcacaagtta  
atgcagtggtaccaacagaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtcctgccagg  
tttagtggcagtggtgtgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
10 aggaaggttcttgacgttcggtggagggaccaagctggaaatcaaacgggggtggcgggtggctcgggcggaggtgggtcgggt  
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atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagtttcttaaaatgaa  
cagctctgcaaactgatgacacagccagataactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa  
15 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
tgggtggaccgtcagttctcttcccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgt  
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
ggaggagcagtaaacagcacgtacctgtgtggtcagcgtcctaccgtctgcaccaggactggctgaatggcaaggagtacaag  
tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt  
20 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcttcttcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctcctgtctccgggtaaacgggacgtcttgaacatggaccattcctggtgctgctgcactcgtgt  
ccggcagcctgtcgggaacgatctgatggagctcaagttctgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgca  
25 gagtggcctggacctgttcacggtgctgctggagcagaacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggc  
ctcgtctgccgacacgatctactgcagcgcctggacgacttcgagggcggggacggcgaccgctgcgccccgggggagggag  
atctgcaggtggcatttgacattgtgtgtgacaatgtggggagagactggaaaagactggccgcgagctgaaggtgtctgagggc  
aagatggatgggattgaggagaagtacccccgaagtctgagtgcgggtaaggagagtgctgaaagtctggaagaatgctgaga  
agaagaacgctcggtggcggactggtcaaggcgtgcggacctgcaggctgaatctggtggctgacctggtggaagaagccc  
30 aggaatctgtgagcaagagtgaataatgtccccagtactaagggttcaactgtgtcttctcagaaacacctgactcagatcgt

Amino acid sequence:

mdfqvqifsfllisasvimrsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfsrgsgtdfslnihpveeddiamyfcqsrkvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit  
35 ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqddtaryycardgysnfh  
yyvmdywgqgtsvtvssdlepkssdkhtspapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwy  
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrdeitkn  
qvsltclvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslsp  
gkadpsnm dpflvllhslsgslsgndlmelkflcrervskrklervqsgldlftvlleqndlerghtgllrellaslrhldllqrlddfea  
40 gtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglivkalrtc  
rlnlvadlveeaquesvksenmspvldstvsstetp

**116. mcasp3-TM/CT**

Nucleotide sequence:

45 Ggaccttcgaacatggagaacaacaaaacctcagtgattcaaatccattaataatttgaagtaaagaccatacatgggagcaag  
tcagtggaactctgggatctatctggacagtagttacaaaatggattatcctgaaatgggcatatgcataataataataagaactcc

ataagagcactggaatgtcatctcgctctggtagcgatgtggacgcagccaacctcagagagacattcatgggcctgaaataccaag  
tcaggaataaaaatgatcttactcgtgaagacattttggaattaatggatagtggttctaagggaagatcatagcaaaaggagcagcttgt  
gtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaatgggcctgtgaactgaaaaagtgactagcttctcagag  
gcgactactgccggagtctgactggaaagccgaaactctcatcattcaggcctgccggggtacggagctggactgtggcattgaga  
5 cagacagtgaggactgatgaggagatggcttgcagaagataccgggtggagggtgacttctgtatgcttactctacagcacctggta  
ctattctggagaaattcaaaggacgggtcgtggttcacccagtcctttgcagcatgctgaagctgtacgcgcacaagctagaatttat  
gcacattctcactcgcgttaacaggaaggtggcaacggaattcagtccttctcctggactccactttccacgcaaaagaaacagatc  
ccgtgtattgttccatgctcacgaaagaactgtactttatcactagctcgagatcgatg

- 10 Amino acid sequence:  
dpsnmennktsvdsksinnfevktihsksvdsgiyldssykmdypemgiciiiiinnknfhkstgmssrsgtdvdaanlretfm  
glkyqvrnkndltredilelmdsvskedhskrssfvclshgdegviyngpvelkktsffrgdyrcsltgkpklfiiqacrgte  
ldcietdsdtdeemacqkipveadflyaystapgyyswrnskdgswfiqslcsmklyahklefihlitrnrvkatefesfsl  
dstfhakkqipcivsmllkelyfyh

15  
**117. 2e12 scFv (SSS-P)H WCH2WCH3-mcasp3-TM/CT**  
Nucleotide sequence:

- aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
20 atgcagtggtaccaacagaaaccaggacagccaccaactcctcatctctgctgcacccaacgtagaatctggggtccctgccagg  
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaagt  
aggaaggttccttggacgttcgggtggaggcaccaagctggaaatcaaacggggtggcgggtggctcgggagggtgggtcgggt  
ggcggcggtatctcagtgacgtgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggtcgcagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
25 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggaactccaagagccaagttttctaaaaatgaa  
cagctctgcaaaactgatgacacagccagataactactgtgccagagatggtatagtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc  
tgggtggaccgtcagtccttcttcccccaaaaaccaaggacacctcatgatctcccggaccctgaggtcacatgcgtggtggt  
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
30 ggaggagcagtaaacagcacgtaccgtgtgtgcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag  
tgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatccaaagccaaagggcagccccgagaaccacaggtgt  
acacctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctcatgctccgtgatgcatgaggctctgcacaac  
35 cactacacgcagaagagcctctcctgtctccgggtaaagcgatccttcgaacatggagaacaacaaacctcagtggaatcaaaa  
tccattaataattttgaagtaaagaccatacatgggagcaagtcagtggtactctgggatctatctggacagtagttacaaaatggattatc  
ctgaaatgggcatatgcataataattaataaagaacttcataagagcactggaatgtcatctcgctctggtaggcatgtggacgca  
gccaacctcagagagacattcatgggcctgaaataccaagtgcaggaataaaaatgatcttactcgtgaagacattttggaattaatgga  
tagtgtttctaaggaagatcatagcaaaaggagcagcttctgtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaa  
40 tgggcctgttgaactgaaaaagttgactagcttctcagaggcgactactgccggagtctgactggaagccgaaactcttcattc  
aggcctgccggggtacggagctggactgtggcattgagacagacagtgaggactgatgaggagatggcttgcagaagataaccggt  
ggaggctgacttctgtatgcttactctacagcacctggttactattcttgagaaattcaaaggacgggtcgtggttcacccagtcctt  
tgcagcatgctgaagctgtacgcgcacaagctagaatttatgcacattctcactcgcgttaacaggaaggtggcaacggaattcagat  
ccttctccttggactccactttccacgcaaaagaacagatcccgtgtattgtgtccatgctcacgaaagaactgtactttatcactagct  
45 cgagatcgatg

Amino acid sequence:

mdfqvqifsflisasvimsgvdivltqspaslavslgqratiscrasesveyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfsqsgsgtdfslnihpveeddiamyfcqqrkvpwtfgggtkleikrgggsgggsgggsgqvlqkesgpglvapsqslsit  
ctvsgfsltygvnwvrqppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh  
5 yyvmdywgqgtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrldeltn  
qvsltclvkgyfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp  
gkadpsnmennktsvdsksinnfevktihgsksvdsgiyldssykmdypemgiciiiinnknfhkstgmssrsgtdvdaanre  
tfmgikyqvmkndltredilelmdsvskedhskrssfvcvilshgdegviygtngpvelkkltffrgdyrcsltgkpklfiiqacr  
10 gteldcgietdsgtdeemacqkipveadflyaystapgyyswrmskdgswfiqslcsmklyahklefmhiltrvrnkatefes  
fslldstfhakkqipcivsmiltkelyfyh

118. **2e12 scFv (SSS-P)H P238SCH2WCH3-mcasp3-TM/CT**

15 Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttcttggctgtgtctctaggtcagagagccaccatctctcgagagccagtgaaagtgtgaatattatgcacaagtta  
atgcagtggtaccaacagaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggctcctgccagg  
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaat  
20 aggaaggttccttggacgttcggtggagggcaccaagctggaaatcaaacggggtggcgggtggctcgggcggaggtgggtcgggt  
ggcggcgagatctcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggtgggaatgatatggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctaaaaatgaa  
cagctctgcaaaactgatgacacagccagatactactgtgccagagatggtatagtaacttcttactatgttatggactactggggtcaa  
25 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
tgggtggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccggacctctgaggtcacatgctgtgtggt  
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaagacaaagccgcg  
ggaggagcagtagacaacagcacgtaccgtgtgttcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag  
tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt  
30 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgtgactccgacggctccttctcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgttctcatgctccgtgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaattcgaacatggagaacaacaaacctcagtgat  
tcaaaatccattaataattttgaagtaaagaccatacatgggagcaagtcagtgactctgggatctatctggacagtagttacaaatg  
35 gattatcctgaaatgggcatatgcataataattaataaagaactccataagagcactggaatgtatctcgtctgtgtacggatgtgg  
acgcagccaacctcagagagacattcatgggcctgaaataccaagtcaggaataaaaatgatcttactcgtgaagacattttggaatta  
atggatagtgttttaaggaagatcatagcaaaaggagcagctttgtgtgtgattctaagccatggtgatgaaggggtcatttatggg  
acaaatgggcctgttgaaactgaaaaagttgactagcttcttcagagggcactactgccggagtctgactggaaagccgaaactctca  
tcattcaggcctgccgggttacggagctggactgtggcattgagacagacagtgaggactgatgaggagatggcttgccagaagata  
40 ccggtggaggctgacttctgtatgcttactctacagcacctggttactattcctggagaaattcaaaaggacgggtcgtggtcatccag  
tccctttgcagcatgctgaagctgtacgcgcacaagctagaatttatgcacattctactcgcgttaacaggaaggtggcaacggaatt  
cgagtccttctccctggactccacttccacgcaaagaacagatcccgtgtattgtgtccatgctcacgaaagaactgtactttatcac  
tagctcgagatogatga

45 Amino acid sequence:

mdfqvqifsfllisasvimsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqsrvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit  
 ctvsgfsltygvnwvrpppgklewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqtddtaryycardgysnfh  
 yyvmdywggqtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
 5 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltkn  
 qvsltlvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslsp  
 gkadpsnsmennktsvdsksinnfevktihgksvdsgiyldssykmdypemgiciniinnknfhkstgmssrgtdvdaan  
 lretfmglkyqvrnkndltredilemdsvskedhskrssfvcvilshgdegviygtngpvelkklsffrgdycrsltgkpklfiiq  
 acrgtelcgiestdsgtdeemacqkipveadflyaystapgyyswrnskdgswfiqslcsmklyahklefmhiltrvrnkivate  
 10 fesfslstdtfhakkipcivsmlltkelyfyh

### 119. mcas8-TM/CT

Nucleotide sequence:

ttcgaacatggattccagagttgtctttatgctattgctgaagaactgggcagtgagacctggctgccctcaagttcctgtgcttgac  
 15 tacatcccacacaagaagcaggagaccatcgaggatgccagaagctatttctgaggctgcgggaaaaggggatgttgagggaag  
 gcaatctgtcttctgaaagagctgctttccacatcagtcggtgggacctgctggtcaactcctagactgcaaccgagaggagatg  
 gtgagagagctgcgggatccagacaatgccagatttctccctacagggatgctctttaaagctctcagaagaagtgcgagttgg  
 aattgagatcttttaagttcctttgaacaatgagatcccaaatgtaagctggaagatgacttgagcctgcttgaaatgttagaatg  
 gagaagaggaccatgctggcagaaaataacttgaaaccctaaaatcaatctgtgaccaggtcaacaagagcctgctggggaagat  
 20 cgaggattatgaaagatcaagcacagagagaagaatgagcctgaaggaagggaagagttgccaccttcagtttgatgagatga  
 gcctcaaatggcggaactgtgtgactcgccaagagaacaagacagtgagtcacggacttcagacaaagttaacaaatgaagaac  
 aaacctcggggatactgtctgatcatcaacaatcatgatttcagcaaggccgggaagacataaccaactccgaaaaatgaaggac  
 agaaaaggaaacagactgtgataaagaggctctgagtaagaccttaaggagcttcatttgagatagtagtcttacgacgactgcactgc  
 aatgaaatccacgagattctagaaggctaccaaaagcgcagaccacaagaacaaagactgctcatctgctgtatctatcccacggt  
 25 gacaagggtgtcgtctatggaacggatgggaaggaggcctccatctatgacctgacatcttacttactggttcaaatgcccttcct  
 gtctgggaaaccaagatcttttcattcaggcttgccaaggaagtaacttcagaaaggagtgctgatgaggcaggcttcagcaa  
 cagaaccacactttagaagtggattcatctcacaagaactatattccgatgaggcagactttctgctgggaatggctacggtgaa  
 gaactgcgttctaccgagatcctgtaatggaacctggtatattcagtcactttgccagagcctgagggaagatgtctcaaggag  
 atgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacaggaggaacaagggaagcagatgccacag  
 30 cccaccttcacactacggaagaagctcttctccctccctaagctcagatcgatt

Amino acid sequence:

snmdfqsclyiaaeelgsedlaalkflclidyphkkqetiedaqklflrlrekgmleegnlsflkellfhisrwdllvnfldcnreem  
 35 vrelrdpdnaqispyrvmlflklseevselelrsfkflinneipckckleddlsleifvemekrtmlaennletlksicdqvnskllgki  
 edyerssterrmslegreelppsvldemslkmaelcdspreqdsesrtsdkvyqmknkprgycliinnhdfskareditqlrkm  
 kdrkgtdcdkealsktfkelhfeivsyddctaneiheilegyqsadhknkdcficcilshgdkgvvygtdgkeasiydltsyftgs  
 kcpslsgkpkiffiqacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrdpvnngtwyiqslc  
 40 qslrercpqqddilsiltgvnydvsnkddrnkgkqmpqptflrkkllffpp

### 120. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcas8-TM/CT

Nucleotide sequence:

aagcttatggatttcaagtgagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacca  
 45 atctccagcttcttggctgtgtctctagtcagagagccaccatctcctcgagagccagtgaaagtgttgatattatgcacaagtta  
 atgcagtggtaccaacagaaaccaggacagccaccaaacctcctcatctctgctgcatccaacgtagaatctggggtccctgccagg

tttagtggcagtgggctctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
 aggaaggttccttgacgttcgggtggaggcaccaagctggaaatcaaacgggggtggcgggtggctcgggagggtgggtcgggt  
 ggcggcggtatcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
 5 atggaagcacagactataattcagctctcaatccagactgagcatcaccaaggacaactccaagagccaagtttctaaaaatgaa  
 cagctctgcaaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtgcaa  
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
 tgggtggaccgtcagttctcttccccccaaaacccaaggacaccctcatgatctccggaccctgaggtcacatgctggtggt  
 ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagccgcg  
 10 ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag  
 tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatcccgggatgagctgaccaagaaccagggtcagcctgacctgcctggtcaaggcttctatcccagcgacatc  
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc  
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctcatgctccgtgatgcatgaggctctgcacaac  
 15 cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggatttcagagttgtctttatgctattgctgaa  
 gaactgggcagtgaaagacctggctgccctcaagttcctgtgcttggactacatcccacacaagaagcaggagaccatcgaggatgc  
 ccagaagctatttctgaggctgcgggaaaaggggatgttggaggaaggcaatctgtcttctgaaagagctgctttccacatcagtc  
 ggtgggacctgctggtcaacttcctagactgcaaccgagaggagatggtgagagagctgcgggatccagacaatcccagatttct  
 ccctacagggtcatgctcttaagctctcagaagaagtgagcgagttggaattgagatctttaagttcctttgaacaatgagatcccca  
 20 aatgtaagctggaagatgacttgagcctgcttgaaatttttagaagaatggagaagaggaccatgctggcagaaaataacttggaac  
 ctaaaatcaatctgtgaccaggtcaacaagagcctgctggggaagatcgaggattatgaaagatcaagcacagagagaagaatga  
 gccttgaagggaagggaagagttgccacctcagtttggatgagatgagcctcaaaatggcggaactgtgtgactcgccaagagaac  
 aagacagtgagtcacggacttcagacaaaagtttaccaaatgaagaacaaacctcggggatactgtctgatcatcaacaatcatgattc  
 agcaaggcccggaagacataaccaactccgaaaaatgaaggacagaaaaggaaacagactgtgataaaggagctctgagtaag  
 25 accttaaggagcttattttgagatagtatcttacgacgactgcactgcaaatgaaatccacgagattctagaaggctaccaaaagcgc  
 agaccacaagaacaaagactgcttcatctgctgtatcctatcccacgggtgacaagggtgtcgtctatggaacggatgggaaggaggc  
 ctccatctatgacctgacatcttacttactggttcaaagtgccttccctgtctgggaaacccaagatcttttcattcaggcttgccaag  
 gaagtaacttcagaaaggagtgctgatgaggcaggttcgagcaacagaaccacactttagaagtggattcatcttcacaaga  
 actatattccggatgaggcagactttctgctgggaatggctacgggtgaagaactgcgttctaccgagatcctgtgaatggaacctgg  
 30 tatattcagtcactttgccagagcctgagggaaagatgtcctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgt  
 gagcaataaagacgacaggaggaacaagggaagcagatgccacagcccaccttcacactacggaagaagctcttctccctccc  
 taatgactcagatcgatt

Amino acid sequence:

35 mdfqvqifsfllisasvimsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgpglvapsqslsit  
 ctvsgfsltgygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqddtaryycardgysnfh  
 yyvmdywggqtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtpevtcvvdvshedpevkfnwy  
 vdgvevhnahtkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltn  
 40 qvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdkswqqgnvfscsvmhleahnhytqkslslsp  
 gkadpsnmfdqsclyiaaelgsedlaalkflcldyiphkkqetiedaqklflrrekgmleegnlsflkellfhisrwdllvnfldc  
 nreemvreldrpdnaqispyrvmlflkseevselelrsfkflnneipkckleddlsleifvemekrtmlaennletlksicdqv  
 kslgkiedyersstermslegreelppsvldemslkmaelcdspreqdsesrtdkvyqmknkprgycliinnhdfskaredi  
 tqlrkmkdrkgtdcdkealsktfkelhfeivsyddctaneiheilegyqsadhknkdcficilshgdkgvvygtdgkeasiydl  
 45 syftgskcpslsgkpkiffiqacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrdpvngrw  
 yiqslcqlsrercpqgddilsiltgvnydvsnkddrrnkkgkmpqptflrkklffpp



**121. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT**

Nucleotide sequence:

5 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca  
atctccagcttctttggctgtgtctctaggtcagagagccaccatctctcgagagccagtgaaagtgtgaatattatgtcacaaagttta  
atgcagtggtagcaacagaaaccaggacagccacccaaactcctcatctctgtcgcacccaacgtagaatctgggggtccctgccagg  
tttagtggcagtggtgtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
aggaaggttccttgagcttcgtggaggacaccaagctggaaatcaaacgggggtggcgggtggtcgggaggaggtgggtcgggt  
ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
10 cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa  
cagctctgcaaactgatgacacagccagataactactgtgccagagatgggtatagtaactttcattactatgttatggactactgggtgcaa  
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatcccaccgtcccagcacctgaactcc  
tgggtggatcgtcagttctcttctccccccaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtggt  
15 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggagggtgcataatgccaagacaaagccgcg  
ggaggagcagtagacaacagcacgtacctgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtagaag  
tgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatccaaagccaaagggcagccccgagaaccacaggtgt  
acacctgcccccatccgggatgagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc  
20 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctccgtgatgcatgaggctctgcacaac  
cactacacgcagaagagcctctcctgtctccgggtaaagcggtaccttcgaacatggatttcagagttgtctttatgctattgctgaa  
gaactgggcagtgaaagacctggctgccctcaagttcctgtgcttgactacatcccacacaagaagcaggagaccatcgaggatgc  
ccagaagctatttctgaggctgcgggaaaaggggatgttgagggaaggcaatctgtcttcttctgaaagagctgctttccacatcagtc  
gggtgggacctgctggtcaacttcctagactgcaaccgagaggagatggtgagagagctgcgggatccagacaatgccagatttct  
25 ccctacaggggtcatgctcttaagctctcagaagaagtgagcgagttggaattgagatcttttaagttccttttgaacaatgagatcccca  
aatgtaagctggaagatgacttgagcctgcttgaaattttgtagaaatggagaaggaccatgctggcagaaaataacttggaaac  
cctaaaatcaatctgtgaccaggtcaacaagacgtgctggggaagatcgaggattatgaaagatcaagcacagagagaagaatga  
gccttgaaaggaagggaaggtgccacctcagttttgatgagatgagcctcaaaatggcggaactgtgtgactcgccaagagaac  
aagacagtgagtcacggacttcagacaaagttaccaaatgaagaacaacacctcggggatactgtctgatcatcaacaatcatgatttc  
30 agcaaggcccggaagacataacccaactccgaaaaatgaaggacagaaaagggaacagactgtgataaagggtcttgagtaag  
acctttaaggagcttcatttgagatagtagtcttacgacgactgcactgcaaatgaaatccacgagattctagaaggctaccaaaagcgc  
agaccacaagaacaaagactgcttcatctgctgtatcctatcccacgggtgacaagggtgtcgtctatggaacggatgggaaggaggc  
ctccatctatgacctgacatcttacttactggttcaagtgcccttcctgtctgggaaacccaagatcttttcattcagggttgccaag  
gaagtaacttcagaaaggagtgacctgatgaggcaggttcgagcaacagaaccacactttagaagtggattcatcatctcacaaga  
35 actatattccggatgaggcagactttctgctgggaatggctacgggtgaagaactcggttctaccgagatcctgtgaatggaacctgg  
tatattcagtcactttgccagagcctgagggaaagatgtcctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgt  
gagcaataaagacgacaggaggaacaagggaagcagatgccacagcccaccttcacactacggaagaagcttcttccctccc  
taatgactcgagatcgattc

40 Amino acid sequence:

mdfqvqifsfllisasvmsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfsghsgsgtdfslnihpveeddiamyfcqqrkvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit  
ctvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslitkdnksqvflkmnslqddtaryycardgysnfh  
yyvmdywgqgtsvtvssdlepkssdkhtsppspapellgssvflfppkpkdtlmisrtpvctcvvdvshedpevkfnwy  
45 vdgevehnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepvytlppsrdeltn  
qvsltlclvkgfypsdiavewesngqpennkytppvldsdgsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslslsp

gkadpsnmfdqsclyaiaelgsedlaalkfclldyiphkkqetiedaqklflrlrekgmleegnlsflkellfhisrwdllvnfldc  
 neemvrelrdpdnaqispyrvmlfklseevselelrsfkflnneipkckleddlslei fvemekrtmlaennletlksicdqv  
 kslkgkiedyerssterrmslegreelpsvldemslkmaelcdspreqdsesrtsdkvyqmknkprgycliinnhdfskaredi  
 tqlrkmdkrkgtdcdkealsktfkelhfeivsyddctaneiheilegyqsadhknkdcficclshgdkgvvygtdgkeasiydl  
 5 syftgskcpslsgkpkiffiqacqgsnfqkvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrdpvngrw  
 yiqslcqlsrercpqgddilsiltgvnydvsnkddrrnkqmpqptflrkkllfpp

## 122. hcas3-TM/CT

10 Nucleotide sequence:

Ggatccttcgaacatggagaacactgaaaactcagtgattcaaaatccattaaaaatttgaaccaaagatcatacatggaagcga  
 atcaatggactctggaatatccctggacaacagttataaatggattatcctgagatgggtttatgtataataattaataagaatttca  
 taaaagcactggaatgacatctcggctcgttacagatgctgatgcagcaaacctcagggaacattcagaaacttgaaatatgaagtc  
 aggaataaaaatgatcttacacgtgaagaaatttggaattgatgcgtgatgtttctaaagaagatcacagcaaaaggagcagttttgt  
 15 tgtgtgcttctgagccatggtgaagaagggaataattttggaacaaatggacctgtgacctgaaaaaataacaaacttttcagagg  
 gatcgtttagaagcttaactgaaaacccaaacttttcattatcaggcctgccgtggtacagaactggactgtggcattgagacaga  
 cagtgggtgatgatgacatggcgtgtcataaaataccagtgaggccgacttctgtatgcatactccacagcacctggtattattct  
 tggcgaaattcaaaaggatggctcctggttcacagtcgctttgtgccatgctgaaacagtatgccgacaagcttgaatttatgcacatt  
 cttaccgggtaaccgaaaggtggcaacagaatttgagtccttttctttgacgctacttttcatgcaagaacagattccatgtattgt  
 20 ttccatgctcaciaaagaactctattttatcactaactcagatcgata

Amino acid sequence:

dpsnmentensvdsksiknlepkiihgsesmdsgisldnsykmdypemglciiinnknfhkstgmtsrsgtdvdaanlretfrn  
 lkyevrnkndltreeivlmrdvskedhskrsvfcvllshgeegii fgtngpvdllkitnffrgdrslrtgkpkllfiiaqrgetld  
 25 cgietdsgvdddmachkipveadflyaystapgyyswrnskdgswwfiqlcamlkqyadklefmhiltvrnkvatfefsfdf  
 atfhakkqipcivsmllkelyfyh

## 123. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcas3-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacca  
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgcacaagtta  
 atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggtccctgccagg  
 tttagtggcagtggtctgggacagacttcagcctcaacatccatctgtggaggaggatgatattgcaatgtatttctgcagcaaa  
 aggaaggttcttgacgttcggtggagggcacaagctggaaatcaaacggggtggcgggtggctcgggaggaggtgggtcgggt  
 35 ggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
 cagggttctcattaaccggctatggtgtaaaactgggtcggcagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
 atggaagcacagactataattcagctctcaaatccagactgagcatcacaagacaactccaagagccaagtttcttaaaaatgaa  
 cagtctgaaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaa  
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
 40 tgggtggaccgtcagcttctcttccccccaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtggt  
 ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcaagacaaagccgcg  
 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaag  
 tgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgctcaaaggcttctatcccagcgacatc  
 45 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
 tctacagcaagctcaccgttggaagaagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac

cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggagaacactgaaaactcagtggttcaaaa  
 tccattaaaaatttggaccaaagatcatacatggaagcgaatcaatggactctggaatatccctggacaacagttataaaatggattat  
 cctgagatgggtttatgtataataattaataagaattttcataaaagcactggaatgacatctcggctctggtacagatgtcgcag  
 caaacctcagggaaacattcagaaacttgaaatatgaagtcaggaataaaaatgatcttacacgtgaagaaatttggaattgatcgt  
 5 gatgtttctaaagaagatcacagcaaaaggagcagttttgtgtgtcctctgagccatggtgaagaaggaataattttggacaaaat  
 ggacctgttgacctgaaaaaataacaaacttttcagaggggagcgtttagaagtcactggaiaacccaaacttttcattattcag  
 gcctgccgtggtacagaactggactgtggcattgagacagacagtggtgtgatgatgacatggcgtgtcataaaataaccagtgagg  
 gccgacttctgtatgcatactccacagcacctggttatttcttggcgaaattcaaaggatggctcctggttcaccagtcgcttgtgc  
 catgctgaaacagtatgccgacaagcttgaatttatgcacattcttaccgggtaaccgaaaggtggcaacagaatttgagtcctttc  
 10 ctttgacgctacttttcagaaagaaacagattccatgtattgtttccatgctcacaaaagaactctattttatcactaactcagatcgc  
 a

Amino acid sequence:

mdfqvqifsflisasvmsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
 15 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggtkleikrgggsgggsgggsgvqlkesgplvapsqslsit  
 ctvsgfsltgygvnwvrppgkglewlgmiwgdgstdynsalksrsltkdnskssqvfikmnsdqtdtaryycardgysnfh  
 yyvmdywgqgtsvtvssdlepkssdkthtspapellggpsvflfppkpkdtlmsrtpevtcvvdvshedpevkfnwy  
 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapiektiskakgpprepqvytlppsrdeltkn  
 qvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahhnytkslslsp  
 20 gkadpsnmentensvdsksiknlepkiihgsesmdsgisldnsykmdypemglciiinnknfhkstgmtsrsgtdvdaanle  
 tfmllkyevmknldtreeivelmrdvskedhskrssfcvllshgeegiiifgtngpvdllkitnffrgdrsrsltkpklfiiqacrgt  
 eldcgietsgvdmdmachkipveadflyaystapgyyswmksdgswwfiqslcamlkqyadklefmhiltrvnrkvatefesf  
 sfdatfhakkqipcivsmlltkelyfyh

## 25 123. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca  
 atctccagcttctttggctgtgtctctaggtcagagagccaccatctctcgagagccagtgaaagtgtgaatattatgcacaagtta  
 atgcagtggtaccaacagaaaccaggacagccaccaaacctctcatctctgctgcacccaacgtagaatctggggtccctgccagg  
 30 ttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaat  
 aggaaggttcttgacgttcggtggagggaccaagctggaatcaaacggggtggcggtggctcggcgagggtgggtcgggt  
 ggcgggcgagatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
 caggggttctattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatggggtg  
 atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagttttctaaaaatgaa  
 35 cagctctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactggggtcaa  
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
 tgggtggatcgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgcgtggtggt  
 ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
 ggaggagcagtagaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaaggagtacaag  
 40 tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaaggcgagccccgagaaccacaggtgt  
 acacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc  
 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
 cactacacgcagaagagcctctccctgtctccgggtaaagcggatccttgaacatggagaacactgaaaactcagtggttcaaaa  
 45 tccattaaaaatttggaccaaagatcatacatggaagcgaatcaatggactctggaatatccctggacaacagttataaaatggattat  
 cctgagatgggtttatgtataataattaataagaattttcataaaagcactggaatgacatctcggctctggtacagatgtcgcag

caaacctcagggaaacattcagaaacttgaaatatgaagtcaggaataaaaatgatcttacacgtgaagaaattgtggaattgatgcgt  
 gatgtttctaagaagatcacagcaaaaggagcagttttgtgtgcttctgagccatggtgaagaaggaataatttttgaacaaat  
 ggacctgttgacctgaaaaaataacaaacttttcagaggggagcgtttagaagtcactggaaaacccaaacttttcattattcag  
 gcctgccgtggtacagaactggactgtggcattgagacagacagtggtgtgatgatgacatggcgtgcataaaataaccagtggag  
 5 gccgacttctgtatgcatactccacagcacctggtattattcttggcgaaattcaaggatggctcctggttcatccagtcgctttgtgc  
 catgctgaaacagtatgccgacaagcttgaatttatgcacattcttaccgggtaaccgaaaggtggcaacagaatttgagtcctttc  
 ctttgacgctacttttcagcaagaaacagattccatgtattgttccatgctcacaaaagaactctattttatcactaactcgagatcgat  
 aa

10 Amino acid sequence:

mdfqvqifsflisasvimsvrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfgggkileikrgggsgggsgggsgqvlksgpplvapsqslsit  
 ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrlsitkdnsksqvflkmnslqddtaryycardgysnfh  
 yyvmdywgqgtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
 15 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeyckvsnkalpapiektiskakgpprepqvylppsrdeltn  
 qvsltlvkgfypsdiavewesngqpennyktpvpldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp  
 gkadpsnmentensvdsksiknlepkiihgsesmdsgisldnsykmndypemglciiinnknfhkstgmtsrsgtdvdaanre  
 tfmlkyevrnkndltreeivelmrdivskedhskrssfcvllshgeegiifgtngpvdllkitnffrgdrsrsltgkpklfiiqacrgt  
 eldcgietsdgvdddmachkipveadflyaystapgyyswrnskdgswfiqslcamlkqyadklefmhiltrvnrkvatefesf  
 20 sfdatfhakkqipciivsmltkelyfhy

**124. hcasp8-TM/CT**

hCaspase8B:

Nucleotide sequence:

25 ggatccttcgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctga  
 gcctggactacattccgaaaggaagcaagaacccatcaaggatgccttgatgtattccagagactccaggaaaagagaatgttg  
 aggaaagcaatctgtccttctgaaggagctgctctccgaattaatagactggatttctgattacctacctaactagaaaggag  
 gagatggaaagggaacttcagacaccaggcagggtcctcaaatctgctacagggtcatgctctatcagatttcagaagaagtgagc  
 agatcagaattgaggtctttaaagttcttttgaagaggaaatctccaaatgcaaactggatgatgacatgaacctgctggatatttcat  
 30 agagatggagaagagggtcatcctgggagaaggaagtggacatctgaaaagagctgtgccccaaatcaacaagagcctgctg  
 aagataatcaacgactatgaagaattcagcaaaagagagaagcagcagcctgaaggaagtcctgatgaatttcaaatggggagga  
 gttgtgtgggtaatgacaatctcgactctcaagagaacaggatagtgaatcacagactttggacaaagttaaccaatgaaaagc  
 aaacctcggggatactgtctgatcatcaacaatcacaatttgcaaaagcacgggagaagtgcccaaaactcacagcattaggagac  
 aggaatggaacacacttgatgcaggggcttgaccacgaccttgagagcttcatttgagatcaagccccacgatgactgcacag  
 35 tagagcaaatctatgagattttgaaaatctaccaactcatggaccacagtaacatggactgcttcatctgctgtatcctctccatggaga  
 caagggcacatctatggcactgatggacaggaggccccatctatgagctgacatctcagttcactggttgaagtgccttccttg  
 ctggaaccacaaagtgtttttattcaggctgtcagggggataactaccagaaaggtatacctgttgagactgattcagaggagcaa  
 ccctatttagaaatggatttatcatcacctcaaacgagatatatcccgatgaggctgactttctgctggggatggccactgtgaataact  
 gtgttccctaccgaaaccctgcagaggggaacctgggtacatccagtcactttgccagagcctgagagagcgtatgcctcgaggcgatg  
 40 atatttcaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagccta  
 ctttcacactaagaaaaaaactgtcttccctctgattgagcatgcatcgata

Amino acid sequence:

45 Dpsnmdfsmlydigeqlsdslaslkflsldyipqrkqepikdalmlfqlrleqrmleesnlslkellfrinrldlitylntrkee  
 merelqtpgraqisayrvmlyqiseevsrslrsfllqeeiskckldddmlldifiemekrvilgegkldilkrvcaqinkslk  
 iindyeefskrssslegspdefsngeelcgvmtisdspreqdsesqldkvyqmkskprgycyliinnhnfakarekvpklhsir

drngthldagaltttfeelhfeikphddctveqiyeilkiyqlmdhsnmndcficilshgdkgiiygtgdqeapiyeltsqftglkcp  
slagkpkvffiqacqgdnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvcsyrnpaegtwyiqslcqlr  
ercprgddiltitvevnsnkddkknmgkqmpqptflrkkklvfpd

5 **hCaspase8C:**

Nucleotide sequence:

ggatccttcgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcctga  
gcctggactacattccgcaaaggaagcaagaacccatcaaggatgccttgatgttattccagagactccaggaaaagagaatgttg  
aggaagcaatctgtccttctgaaggagctgtcttccgaattaatagactggatttctgattacctacctaactagaaaggag  
10 gagatggaaaggggaacttcagacaccaggcagggtcctcaatttctgcctacagggtcatgtctatcagatttcagaagaagtgagc  
agatcagaattgaggtcttttaagtttctttgcaagaggaaatctccaaatgcaaactggatgatgacatgaacctgctggatatttcat  
agagatggagaagagggtcatcctgggagaaggaaagtggacatcctgaaaagagtctgtgcccaaatcaacaagagcctgctg  
aagataatcaacgactatgaagaattcagcaaaggggaggagtgtgtggggtaatacacaatctcggactctccaagagaacaggat  
agtgaatcacagactttggacaaagtttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaattttgcaa  
15 agcacgggagaaagtgcccaaacttcacagcattagggacaggaatggaacacacttgatgcaggggctttgaccacgacctttg  
aagagcttcattttagatcaagccccagatgactgcacagtagagcaaatctatgagatttgaaaatctaccaactcatggaccac  
agtaacatggactgcttcatctgtgtatcctctcccatggagacaagggcatcatctatggcactgatggacaggaggccccatcta  
tgagctgacatctcagttcactggttgaagtgcccttcccttctggtgaaaacccaaagtgtttttattcagcctgtcagggggataact  
accagaaaggatatacctgttgagactgattcagaggagcaaccctattagaaatggatttatcatcacctcaaacgagatatatccc  
20 gatgaggctgactttctgtggggatggccactgtgaataactgtgttctaccgaaacctgcagagggaacctggtacatccagt  
cactttgccagagcctgagagagcgtatcctcaggcgatgatattctcaccatcctgactgaagtgaactatgaagtaagcaacaa  
ggatgacaagaaaaacatggggaaacagatgcctcagcctactttcacactaagaaaaaaacttgtctcccttctgattgagcatgca  
tcgata

25 Amino acid sequence:

dpsnmndfsmlydigeqlsdslaslkflsldyipqrkqepikdalmlfqlrleqrmleesnlslfkellfrinrldlitylntrkeem  
erelqtpraqisayrvmllyqiseevrselrsfkflqeeiskcklddmnlldifiemekrvilgegkldilkrvcaqinkslkii  
ndyeefskgeelcgvmtisdspreqdsesqtlldkvyqmkskprgycliinnhnfakarekvpklhsirdngthldagaltttfe  
lhfeikphddctveqiyeilkiyqlmdhsnmndcficilshgdkgiiygtgdqeapiyeltsqftglkcpslagkpkvffiqacqg  
30 dnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvcsyrnpaegtwyiqslcqlrercprgddiltitvev  
evnsnkddkknmgkqmpqptflrkkklvfpd

**125. 2e12 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp8-TM/CT**

Nucleotide sequence:

aagcttatggattttcaagtgagattttcagcttctgctaatcagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
35 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaagtggtgaatattatgcacaagttta  
atgcagtggtagcaacagaaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctgggggtccctgccagg  
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
aggaaggttccctggacgttcggtggaggcaccagctggaaatcaaacggggtggcggtggctcggggcggagggtgggtcgggt  
40 ggcggcggtatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggtg  
atggaagcacagactataattcagctctcaaatccagactgagcatccaaggacaactccaagagccaagttttctaaaaatgaa  
cagtctgcaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggggtcaa  
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacatccccaccgtccccagcacctgaactcc  
45 tgggtggaccgtcagttcttcttcccccaaaaccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggt  
ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg

ggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaag  
 tgcaaggtctccaacaagccctcccagccccatcgagaaaacctctcaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatccgggatgagctgaccaagaaccagggtcagcctgacctgctggtcaaaggcttctatcccagcgacatc  
 gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
 5 tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
 cactacacgcagaagagcctctccctgtctccgggtaagcggatcctcgaacatggacttcagcagaaatcttatgatattgggga  
 acaactggacagtgaagatctggcctccctcaagttcctgagcctggactacattccgcaaaggaagcaagaacctatcaaggatg  
 ccttgatgttattccagagactccaggaaaagagaatgttgagggaagcaatctgctcctgaaggagctgcttccgaattaata  
 gactggatttgctgattacctaactagaagaggagatggaaaggggaacttcagacaccaggcagggctcaaatttctg  
 10 cctacagggctcatgctctatcagatttcagaagaagtgagcagatcagaattgaggtctttaagtttctttgcaaggaggaaatctcaa  
 atgcaaactggatgatgacatgaacctgctggatatttcatagagatggagaagaggggtcatcctgggagaaggaaagttggacat  
 cctgaaaagagctgtgcccacaatacaagagcctgctgaagataataacgactatgaagaattcagcaaagagagaagcagca  
 gccttgaaagagctcctgatgaatttcaaaggggaggagtgtgtgggtaatacacaatctcggactctcaagagaacaggatag  
 tgaatcacagactttggacaaagttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaatttgcaaaag  
 15 cacgggagaaagtgcccaaacttcacagcattagggacaggaatggaacacacttgatgcaggggctttgaccacgaccttgaa  
 gagcttcattttgatcaagccccacgatgactgcacagtagagcaaatctatgagatttgaaaatctaccaactcatggaccacag  
 taacatggactgcttcatctgtgtatcctctccatggagacaagggcatcatctatggcactgatggacaggaggccccatctatg  
 agctgacatctcagttcactggttgaaagtgcccttccctgctggaaaacccaaagtgtttttattcaggcttgcagggggataactac  
 cagaaaggatatacctgttgagactgattcagaggagcaaccctatttagaaatggatttatcatcacctcaaacgagatatatccggat  
 20 gaggtgactttctgctggggatggccactgtgaataactgttttctaccgaaaccctgcagagggaacctggtacatccagtcact  
 ttgccagagcctgagagagcgtatgtctcaggcgatgatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggat  
 gacaagaaaaacatggggaaacagatgcctcagcctactttcacactaagaaaaaactgtcttcccttctgattgagcatgcatcga  
 ta

25

Amino acid sequence:

mdfqvqifsflisasvmsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppklisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqrkvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit  
 ctvsgfsltgygvnwvrpppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh  
 30 yyvmdywggqtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvytlppsrdeltkn  
 qvsltlvkgfypsdiavewesngqpennyktpvpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsps  
 gkadpsnmdfsmlydigeqlsdslaskflsldyipqrkqepikdalmflqrlqekrmleesnlslfcellfrinrldlitylntrk  
 eemerelqtpgraqisayrvmllyqiseevsrslsfklqeeiskckldddnnlldifiemekrvilgegkldilkrvcaqinksl  
 35 lkiindyeefskerssslegspdefsngeelcgvmtisdspreqdsesqtdkvyqmkskprgycliinnhnfakarekvpklhsi  
 rdngthldagalttfeelhfeikphddctveiqiyeilkiyqlmdhsnmddficilshgdkgiiygtgdqeapiyeltsqftglkc  
 pslagkpkvffiqacqgdnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnncvsyrnpaegtwyiqslcql  
 rercprgdiltilevnyevsnkddkknmgkmpqptflrkklvfspd

40

# **126. 2e12 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp8B-TM/CT** **(other caspase 8 isoforms are similar)**

Nucleotide sequence:

aagcctatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacca  
 45 atctccagcttctttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgttgatattatgcacaagtta  
 atgcagtggtagcaacagaaccaggacagccaccaaacctcctcatctctgctgcatccaacgtagaatctggggctcctgccagg

tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
 aggaaggttccttggacgttcggtggagggaccaagctggaaatcaaacggggtggcgggtggctcgggagggtgggtcgggt  
 ggcggcggtatcaggtgcagctgaaggagtcaggacctggcctggtggcgcctcacagagcctgtccatcacatgcaccgtct  
 cagggttctcattaaccggctatggtgtaaactgggtcggcagcctccaggaaagggtctggagtggtgggaatgatatggggtg  
 5 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaaatgaa  
 cagtctgcaaaactgatgacacagccagataactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtgcaa  
 ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaactcc  
 tgggtggatcgtcagttcttcttcccccaaaaacccaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggt  
 ggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcg  
 10 ggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag  
 tgcaaggtctccaacaaagccctccagcccccatcgagaaaaccatctcaaagccaaagggcagccccgagaaccacaggtgt  
 acacctgccccatcccggtgatgagctgaccaagaaccagggtcagcctgacctgcctggctcaaaggcttctatcccagcgacatc  
 gccgtggagtgaggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcc  
 tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgtcttctcatgctcctgatgcatgaggctctgcacaac  
 15 cactacacgcagaagagcctctcctgtctccgggtaaagcggatccttcgaacatggacttcagcagaaatcttatgatattgggga  
 acaactggacagtgaagatctggcctccctcaagttcctgagcctggactacattccgaaaggaaagcaagaacctatcaaggatg  
 ccttgatgttattccagagactccaggaaaagagaatgttgaggaaagcaatctgcttctcgaaggagctgctctccgaattaata  
 gactggatttctgattacctaactaactagaaaggaggagatggaagggaacttcagacaccaggcagggtcctcaatttctg  
 cctacagggtcatgctctatcagattcagaagaagtgagcagatcagaattgaggtctttaagttcttttgaaggaggaaatctccaa  
 20 atgcaaaactggatgatgacatgaacctgctggatatttcatagagatggagaagagggtcatcctgggagaaggaaagtggacat  
 cctgaaaagagctgtgccccaaatcaacaagagcctgctgaagataatcaacgactatgaagaattcagcaagagagaagcagca  
 gccttgaagggaagtcctgatgaatttcaaatggggaggagtgtgtggggtaatgacaatctcggactctccaagagaacaggatag  
 tgaatcacagactttggacaaagttaccaaatgaaaagcaaacctcggggatactgtctgatcatcaacaatcacaatttgcaaaag  
 cacgggagaaagtgccaaacttcacagcattagggacaggaatggaacacacttgatgcaggggcttgaccacgaccttgaa  
 25 gagcttcattttgagatcaagccccacgatgactgcacagtagagcaaatctatgagattttgaaaatctaccaactcatggaccacag  
 taacatggactgctcatctgctgtatcctctcccatggagacaagggtcatcatatggcactgatggacaggaggcccccatctatg  
 agctgacatctcagttcactggttgaaagtcccttccctgctggaaaacccaaagtgtttttattcaggcttgcagggggataactac  
 cagaaaggatatacctgttgagactgattcagaggagcaaccctatttagaaatggattatcatcacctcaaacgagatatatccggat  
 gaggtgacttctgctggggatggccactgtgaataactgtgttctaccgaaacctgcagagggaacctgtacatccagtcact  
 30 ttgccagagcctgagagagcgtatgctcagggcgatgatattctcaccatcctgactgaagtgaactatgaagtaagcaacaaggat  
 gacaagaaaaacatggggaacagatgcctcagcctactttcacactaagaaaaaaactgtcttcccttctgattgagcatgcatcga  
 taa

35 Amino acid sequence:  
 mdfqvqifsfllisasvimrsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyyqqkpgqppkllisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqrkvpwtfgggtkleikrgggsgggsgggsgggsgvqlkesgpglvapsqslsit  
 ctvsfsltygvnwvrpppgkglewlgmiwgdstdynsalksrslitkdnksqvflkmnslqtdtdaryycardgysnfh  
 yyvmdywgqgtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwy  
 40 vdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlpssrdeltkn  
 qvsltlvkgfypsdiavewesngqpennyktppvldsdgsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslslsp  
 gkadpsnmfdsrnlydigeqlsdslaslkflsldyipqrkqepikdalmlfqlqekrmleesnlslkellfrinrldlitylntrk  
 eemerelqtpgraqisayrvmllyqiseevsrslrsfkflqeeiskekllddmnlldifiemekrvilgeglkdlkrvcaqinksl  
 lkiindyeefskerssslegspdefsngeelcgvmtisdspreqdsesqtdkvyqmkskprgycliinnhnfakarekvpklhsi  
 45 rdmngthldagaltttfeelhfeikphddctveqiyelkiyqlmdhsnmddcficilshgdkgiygtgdgeapiyeltsqftglkc

pslagkpkvffiqacqgdnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvsvyrnpaegtwyiqslcql  
rercprgdiltilevnyevsnkddkknmgkqmpqptflrklvfpsd

5 **127. hFADD-TM/CT**

Nucleotide sequence:

Gttgtgataccttcgaacccgttctggtgctgctgcactcgggtgctgctccagcctgtcgagcagcgagctgaccgagctcaagtcc  
tatgcctcggggcgctgggcaagcgcaagctggagcgcgtgcagagcggcctagacctcttccatgctgctggagcagaacga  
cctggagccccgggcacaccgagctcctgcgcgagctgctgcctccctgcggcgccacgacctgtcgggcgctcgacgactt  
10 cgagggcgggggcgggcgccggggcgccgctggggaagaagacctgtgtgcagcattaacgtcatatgtgataatgtggggaa  
agattggagaaggctggctcgcagctcaaagtctcagacaccaagatcgacagcatcgaggacagataacccccgaacctgaca  
gagcgtgtcggggagtcactgagaatctggaagaacacagagaaggagaacgaacagtggtggccacctggtgggggctctcag  
gtcctgccagatgaacctggtggctgacctggtacaagaggttcagcaggcccgtagacctccagaacaggagtggggccatgtcc  
ccgatgtcatggaactcagacgcacatctacctccgaagcgtcctgataactcgagatcgataacaac

15

Amino acid sequence:

Dpsnpflvllhsvssslsssteltelkflclgrvgrklervqsgldfsmllleqndlepghtellrellaslrhdllrrvddfeagaaag  
aapgeedlcaafnvicdnvgkdwrrlarqlkvsdtkidsiedryprmltervreslriwntekenatvahlvgalrscqmnlvad  
lvqevqqardlqnrsgamspmswnsdastseas

20

**128. 2e12 scFv-hIgG1 (SSS-P)H WCH2 WCH3-hFADD-TM/CT**

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcaccca  
atctccagcttctttggctgtgtctctagtcagagagccaccatctctcgagagccagtgaaagtgtgaatattatgcacaagtta  
25 atgcagtgttaccacagaaaccaggacagccacccaaactcctcatctctgtcgcacccaacgtagaatctgggggtccctgccagg  
tttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaagt  
aggaaggttcttgacgttcgggtggagggcaccaagctggaaatcaaacggggtggcggtggctcgggcggaggtgggtcgggt  
ggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
cagggttctcattaaccggctatggtgtaaactgggttcgccagcctccaggaaagggtctggagtggctgggaatgatatgggggtg  
30 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttcttaaaatgaa  
cagctctgaaaactgatgacacagccagatactactgtgccagagatggttatagtaactttcattactatgttatggactactgggtcaa  
ggaacctcagtcaccgtctcctcagatctggagcccaaatcttctgacaaaactcacacatccccaccgtccccagcacctgaaactcc  
tgggtggaccgtcagcttctcttccccccaaaacccaaggacaccctcatgatctcccggacccttgaggtcacatgcgtggtggt  
ggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcggtggaggtgcataatgcaagacaaagccgcg  
35 ggaggagcagtaaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaag  
tgcaaggctccaacaaagccctcccagcccccatcgagaaaaccatctccaagccaaagggcgagccccgagaaccacaggtgt  
acacctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatc  
gccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttctcc  
tctacagcaagctcaccgtggacaagagcaggtggcagcagggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac  
40 cactacacgcagaagagcctctccctgtctccgggtaagcggatccttcgaacccgttctggtgctgctgcactcgggtgctgcca  
gcctgtcgagcagcgagctgaccgagctcaagttcctatgcctcgggcgctgggcaagcgcaagctggagcgcgtgcagagcg  
gcctagaccttcttccatgctgctggagcagaacgacctggagccccgggcacaccgagctcctgcgcgagctgctgcctccctg  
cggcgccacgacctgctcgggcgctcgacgacttcaggcggggggcgccggcgccggggccgcgcctggggaagaagacctgt  
gtgcagcatttaacgtcatatgtgataatgtggggaagattggagaaggctggctcgtcagctcaaaagtctcagacaccaagatcga  
45 cagcatcgaggacagataacccccgaacctgacagagcgtgtcggggagtcactgagaatctggaagaacacagagaaggaga  
acgcaacagtggtggccacctggtgggggctctcaggtctgccagatgaacctggtggctgacctggtacaagaggttcagcaggc



ccgtgacctccagaacaggagtggggccatgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcga  
gatcgataacaac

Amino acid sequence:

5 mdfqvqifsflisasvimsvrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
arfgsgsgtdfslnihpveeddiamyfcqqsrkvpwtfggggtkleikrgggsgggsgggsgggsgvqlkesgpglvapsqslsit  
ctvsgfsltygvnwvrqppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh  
yyvmdywgqgtsvtvssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtpcvvvdvshedpevkfnwy  
vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrldeltn  
10 qvsltlvkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsisp  
gkadpsnpflvllhsvssslssselteklfclgrvgkrklervqsgldlfsmlleqndlepghtellrellaslrhldllrvddfeagaa  
agaapgeedlcaafnvicdnvgkdwrrlarqlkvsdtkidsiedryprnltervreslriwkntekenatvahlvgalrscqmnlv  
adlvqevqqardlqnrsgamspmswnsdastseas

# 15 129. 2e12 scFv-hIgG1 (SSS-P)H P238SCH2 WCH3-hFADD-TM/CT

Nucleotide sequence:

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcaccca  
atctccagcttctttggctgtgtctctaggtcagagagccaccatctctcagagccagtgaaagtgtgaatattatgcacaagtta  
atgcagtggtaccaacagaaaccaggacagccacccaaactcctcatctctgctgcatccaacgtagaatctggggtccctgccagg  
20 tttagtggcagtggtctgggacagactcagcctcaacatccatctgtggaggaggatgatattgaatgtattctgtcagcaaagt  
aggaaggttccttgacgttcggaggagccaccaagctggaaatcaaacggggtggcgggtggctcgggaggagtggtgcgggt  
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25 cagctctgaaaactgatgacacagccagatactactgtgccagagatgggtatagtaactttcattactatgttatggactactggggtcaa  
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35 gcctgtcagcagcgagctgaccgagctcaagttcctatgcctcgggcgcgtgggcaagcgcaagctggagcgcgtgcagagcg  
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cagcatcgaggacagataccccgcacactgacagagcgtgtgcgggagtcactgagaatctggaagaacacagagaaggaga  
40 acgcaacagtggtggccacctggtgggggtctcaggtctgccagatgaacctggtgggtgacctggtacaagaggttcagcagggc  
ccgtgacctccagaacaggagtggggccatgtccccgatgtcatggaactcagacgcatctacctccgaagcgtcctgataactcga  
gatcgataacaac

45 Amino acid sequence:

mdfqvqifsflisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkillisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqsrvpwtfgggtkleikrgggsgggsgggsgqvlkesgpglvapsqslsit  
 ctvsgfsltygvnwvrqppgkglewlgmiwgdgstdynsalksrslsitkdnksqvflkmnslqtddtaryycardgysnfh  
 yyvmdywggqtsvtvssdlepkssdkthtspspapellggssvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwy  
 5 vdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltkn  
 qvsltlvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslsp  
 gkadpsnplvllhsvssslsssetelkflclgrvgkrklervqsgldlfsmlleqndlepghtellrellaslrhdllrrvddfeagaa  
 agaapgeedlcaafnvidcnvgkdwrrlarqlkvsdtkidsiedryprnltervreslriwkntekenatvahlvgalrscqmnlv  
 adlvqevqqardlqnrsgamspmswnsdastseas

10

**130. 1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-hCD80TMCT**

Nucleotide sequence

aagcttatgattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagctccagttaagttacatgtactgtgtac  
 15 cagcagaagtcagcgcctccccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccatttactgtcagcagtgaggtagtactccgc  
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtggcggcgatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggctggaatggatgggaataatatattatgatggaggcacagatt  
 20 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaaatcaacagctgcaaactgat  
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 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctggggggatcgtcagttcttcttccccccaaaaccca  
 aggacaccctcatgatctcccgaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagaccctgaggtcaagttcaac  
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 25 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcga  
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaa  
 ccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgaggagagcaatggcgagccggagaac  
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 gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaa  
 30 agcggatccttcgaacctgctccatctgggccattacctaatactcagtaaatggaattttgtgatatgctgcctgacctactgctttg  
 cccaagatgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgtataaatcgat

Amino acid sequence:

mdfqvqifsflisasvimsrgvdivltqspptiaaspgekvittrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
 35 sgsgtsyslaintmetedaatyycqqsstpltfsgstkleikrgggsgggsgggsgqvlkeagpglvqptqtlstctvsgfs  
 ltsdgvhwirppgkglewmgiyydggt dynsaiksrslsrdtsksqvflkinslqtddtamyycaihfdywgqgvmvtv  
 ssdlepkssdkthtspspapellggssvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree  
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltlvkgfypsdiav  
 40 ewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkadpsnllpswaitlis  
 vngifvicltycfaprerrrrerlrresvrpv

**131.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hCD80TM/CT**

Nucleotide sequence

45

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc  
 atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagttta  
 atgcagtggtaccaacagaaccaggacagccacccaaactcctcatctctgctgcacccaacgtagaatctggggctcctgccagg  
 ttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtatttctgtcagcaaagt  
 5 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac  
 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
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 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtggcggcggtatctc  
 10 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
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 accaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaa  
 20 caactacaagaccacgcctccgtgctggactccgacggctccttctcctctacagcaagctcaccgtggacaagagcaggtggc  
 agcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggta  
 aagcggatccttgaacctgctcccatctgggccattacctaatactcagtaaatggaatgttgatgctgcctgacctactgctt  
 gccccaatgagcagagagagaaggaggaatgagagattgagaagggaaggtgtacgcctgtataaatcgat

25 Amino acid sequence:  
 mdfqvqifsfllisasvimsgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
 arfsgsgsgtdfslnihpveeddiamyfcqqsklmdfqvqifsfllisasvimsgvdivltqspttiaaspgekvtitcrassvsy  
 mywyqqksgasplkiwydtsklasgvpnrfsgsgsgtsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsggg  
 gsgggsgsvqlkeagpqlvqptqlslctvsgfsltsdgvhwirpppgkglewmgiyydgtdynsaiksrlsisrdsksqvf  
 30 lkinslqtdtdamyycaihfdywggvmvtvssdlepkssdkhtsppspapellggspsvflfppkpkdtlmisrtpetcvv  
 vdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeyckvsnkalpapietkiskakgqpr  
 epqvytlppsrdeltknqvsltclvkgyfypsdiavewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvm  
 healhnhytqkslslspgkadsnllpswaitlisvngifvicltycfaprerrrrerlrresvrpv

35  
**132.1D8 scFv mIgAT4-hCD80TM/CT**  
 Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac  
 40 cagcagaagtcaggcgcctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtggcggcggtatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 45 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctctgcaaactgat  
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatcacatctgttctc

ctctactactctctccaccttctgccagcccagcctgtcactgcagcgccagctcttgaggacctgctcttgggttcagatgcc  
agcatcacatgtacttgaatggcctgagagatcttgaggagctgtcttcacctgggagccctccactgggaaggatgcagtgcag  
aagaaagctgtgcagaattcctgcggctgtacagtgtgtccagcgtcctgacctggtgtgctgagcgtggaacagtggcgcatca  
ttcaagtgcacagttaccatcctgagctgtacaccttaactggcacaattgccaaagtcacagtgaacaccttcccacccaggtcca  
5 cctgctaccgccgccgtcggaggagctggccctgaatgagctcgtgtccctgacatgcctgggtgcgagcttcaaccctaaagaagt  
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gccaccacctacctggtgacaagcgtgtgtgctgtatcagctgaaatctggaaacagggtgaccagtactcctgcatggtgggccac  
gaggccttgccatgaacttcaccagaagaccatcgaccgtctgtcgggtaaacccaccaatgtcagcgtgtctgtgatcatgtcag  
agggagaggatccttgaacaacctgtcccatcctgggccattaccttaatctcagtaaatggaattttgtatgtctgcctgacctta  
10 ctgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaaggggaaagtgtacgccctgtataaatcgatact

**Amino acid sequence:**

mdfqvqifsfllisasvimsrqdivltqspptiaaspgekvititrasssvsymwyqqksgasplkiwydtsklasgvpnrfsg  
sgsgtsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsgggsgggsgqvlkeagpglvqptqtsltctvsgfs  
ltsdgvhwirpppgkglewmgiyydgtdynsaiksrlsirdtsksqvfkinslqtddtamyycaihfdywgqgvmtv  
ssdhicspptppppscqpslsqrpaedlllgdsasitctlnglrdpegavftwepstgkdavqkkavqnsqgcysvssvlpgca  
erwnsgasfkctvthpesdtltgtiaktvntfppqvhlpppseelalnelvslctlvrafnpkevlvrwlhgneelspesylvfep  
lkepeggattylvtsvlrvsaeiwbkqgdqyscmvghealpmnftqktidrlsgkptnsvsvimsegedpsnnllpswaitlisv  
ngifviccltycfaprcrerrrnerlrresvrpv

## 133.1D8 scFv hIgE-hCD80-TM/CT

### Nucleotide sequence

aagcttatggatttcaagtgacagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcacta  
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactggtac  
cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
ggcttgggacctcttattctctcgcaatcaacaccatgggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcggtggctcgggcggtgggtgggtcgggtggcgccggatctc  
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatatgactgtctctgggttctcattaa  
ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
ataattcagcaattaaatccagactgagcatcagcaggggacacctccaagagccaagttttctaaaaatacaacagctctgcaaactgat  
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtgtcacagtctcctctgatcacgtctgtctc  
agggacttcacccgccaccgtgaagatcttacagtcgtctgcgacggcgggcgaacttcccccgaccatccagctcctgtg  
cctcgtctctgggtacacccagggactatcaacatcacctggctggaggacgggcaggtcatggacgtggacttgtccaccgcctc  
taccacgcaggagggtgagctggcctccacaaaagcgagctcacctcagccagaagcactggctgtcagaccgcacctacac  
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taagccggccagcccgttcgacctgttcatccgcaagtcgccacgatcacctgtctggtggtggacctggcaccagcaagggg  
accgtgaacctgacctggtcccgggccagtgggaagcctgtgaaccactccaccagaaaggaggagaaagcagcgcaatggcac  
gttaaccgtcacgtccacctgcccgggtgggcacccgagactggatcaggggggagacctaccagtgcagggtgaccaccccca  
cctgccagggccctcatgcggtccacgaccaagaccagcggcccgctgctgccccggaagtctatgcgtttgcgacgccgga  
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gtccagcagcgggtgtctgtaaatccggtaaagcggatccttcgaagctcccatcctgggccattaccttaatctcagtaaatggaat  
tttgtgatatgctgcctgacctactgctttgccccaaagatgcagagagagaaggagggaatgagagattgagaaggggaaagtgtacg  
ccctgtataaatcgata

45

Amino acid sequence:

mdfqvqifsflisavimsrgvdivltqspttiaaspgkvitcrasssvsymywyqqksgasplkiwydtsklasgvpnrfsg  
sgsgtsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs  
ltsdgvhwirpppgklewmgiyydggt dynsaiksrlsirdtsksqvflikslqtdtdamyycaihfdywgqgvmvtv  
5 ssdhvcsrdftptvkilqsscdggghfpptiqlclvsgytpgtinitwledgqvmdivl stasttqegelastqseltlsqkhwls  
drtytcqvt yqghtfedstkkcadsnprgvsaylsrpsfdlfrksptitclvdlapskgtvnlwsrasgkpvnhstrkeekqm  
gtltvtstlpvgtrdwiegetyqcrvthphlpralmrsttksgpraapevyafatpewpgsrdkrtlacliqnfmpe disvqwlhn  
evqlpdarhsttqprktksgffv fsrlevtraeweqkdeficravheaaspsqtvqravsvnpgkadpsklpswaitlisvngifv  
iccltycfapr crerrmerlrresvrpv

10

### 134.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mFADD-TM/CT

Nucleotide sequence

15 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactgggtac  
cagcagaagtcaggcgctcccctaaactctggattatgacacatccaagctggcttctggagttccaaatcgcttcagtgccagtg  
ggcttgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcggcggtggtgggtcgggtggcgccggatctc  
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggtctcattaa  
20 ccagcgatgggtgactgactggattcagagcctccaggaaagggctggaatggatgggaataatatattatgatggaggcacagatt  
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctctgcaactgat  
gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcattggtcacagtctcctctgatctggagcccaa  
atctctgacaaaactcacatccccaccgtcccagcacctgaactcctgggtggaccgtcagctctcctctccccccaaaaccca  
aggacacctcatgatctcccgaccctgaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaac  
25 tggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagc  
gtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtctccaacaaagccctcccagccccatcga  
gaaaacctctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa  
ccaggtcagcctgacctgctggtcaaaaggcttctatccagcgacatcgccgtggagtgaggagcaatgggcagccgggagaac  
aactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagtcaccgtggacaagagcaggtggca  
30 gcagggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaacctacacgcagaagagcctctcctgtctccgggtaa  
agcggatccttcgaacatggaccattctctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctcaa  
gttctgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacgggtgctgctggagcag  
aacgacctggagcgcgggcacaccgggctgctgcgcgagtgctggcctcgtgcgccgacacgatctactgcagcgcctggac  
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35 ggagagactggaaaagactggcccgagctgaaggtgtctgagccaagatggatgggattgaggagaagtacccccgaagtc  
tgagtgagcgggtaaggagagtgtaaagtctggaagaatgctgagaagaagaacgctcgggtggccggactggtcaaggcgc  
tgcggacctgcaggtgaatctggtggctgacctggtggaagaagcccaggaatctgtgagcaagagtgagaatatgtccccagta  
ctaagggttcaactgtgtcttctcagaaacacctgactcgagatcgat

40 Amino acid sequence:

mdfqvqifsflisavimsrgvdivltqspttiaaspgkvitcrasssvsymywyqqksgasplkiwydtsklasgvpnrfsg  
sgsgtsyslaintmetedaatyycqqwsstpltfsggtkleikrgggsgggsgggsgggsgvqlkeagpglvqptqlslctvsgfs  
ltsdgvhwirpppgklewmgiyydggt dynsaiksrlsirdtsksqvflikslqtdtdamyycaihfdywgqgvmvtv  
ssdlepkssdkthtspspapellggs vflfpkp kdtlmisrtp evtcvvvdvshedpevkfnwyvdgvevhnaktkpree  
45 qynstyrvsvlvtlhqdwlngkeyckvsnkalpapiektiskakgpprepqv tylppsrdeltknqvs ltlvkgyf psdiav  
ewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksls lpspgkadpsnmdpflvllhs

lsgslsgndlmelkflcrervskrklervqsgldlftvllleqndlerghtglrellaslrhdllqrlddfeagtataappgeadlqvaf  
divcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglvkalrtcrlnlvadlveeaquesvs  
ksenmspvlrdstvsstetp

5    **135.1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mFADD-TM/CT**

Nucleotide sequence

aagcttatggatttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagtacatgtactggtac  
cagcagaagtcaggcgctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
10    ggtctgggacctcttattctctcgcaatcaacaccatggagactgaagatgctgccacttattactgtcagcagtgtagtagtactccgc  
tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcggcggtggtgggtcgggtggcgggcggtatctc  
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa  
ccagcgatggtgtacactggattcgacagcctccaggaaagggctggaatggatgggaataatatattatgatggaggcacagatt  
ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttctaaaaatcaacagctcgaactgat  
15    gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagctcctctgactcggagcccaa  
atcttctgacaaaactcacatccccaccgtccccagcacctgaactcctgggtggatcgtcagcttctcttccccccaaaaccca  
aggacacctcatgatctccggaccttgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaac  
tggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagc  
gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtcctcaacaaagccctcccagccccatcga  
20    gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaa  
ccaggtcagcctgacctgctgctcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatggcgagccggagaac  
aactacaagaccacgctcccgtgctggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca  
gcaggggaacgtcttctcatgctccgtgatgcatgaggtcttgcacaaccactacacgcagaagagcctctcctgtctccgggtaa  
agcggatccttgaacatggaccattctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatctgatggagctcaa  
25    gttcttgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggcctggacctgttcacggtgctgctggagcag  
aacgacctggagcgcgggcacaccgggctgctgcgcgagttgctggcctcgtgcgccgacacgatctactgcagcgcctggac  
gacttcgagggcggggacggcgaccgctgcggcccgggggaggcagatctgcaggtggcatttgacattgtgtgacaatgtgg  
ggagagactgaaaagactggccgcgagctgaaggtgtctgagccaagatggatgggattgaggagaagtacccccgaagtc  
tgagtgcgagggttaaggagagctgaaagtctggaagaatgctgagaagaagaacgcctcgggtggccggactggtcaaggcgc  
30    tgcggacctgcaggtgaatctggtggctgacctggtggaagaagccaggaatctgtgagcaagagtgagaatatgtccccagta  
ctaagggatcaactgtgtcttctcagaaacacctgactcgagatcgat

Amino acid sequence:

mdfqvqifsfllisasvmsrgvdivltqspttiaaspgekvitcrasssvsmywyqqksgasplwiydtsklasgvpnrfsg  
35    ssgtsyslaintmetedaatyycqwsstpltfsgtkleikrgggsgggsgggsgsqqvqlkeagpglvqptqtlstctvsgfs  
ltsdgvhwirpppgklewmgiiyydggttdynsaiksrslsrdtsksqvfkinslqtdtdamyyecarihfdywgqgvmvtv  
ssdlepkssdkhtspspapellggssvflfppkpkdtlmsirtpcvvvdvshedpevkfnwyvdgvevhnaktkpree  
qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltlvkgyfypsdiav  
ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgkadsnmdpflvllhs  
40    lsgslsgndlmelkflcrervskrklervqsgldlftvllleqndlerghtglrellaslrhdllqrlddfeagtataappgeadlqvaf  
divcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknaekknasvaglvkalrtcrlnlvadlveeaquesvs  
ksenmspvlrdstvsstetp

45    **136.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp3-TM/CT**

Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagttacatgtactggtag  
 cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacccatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc  
 5 tcacgttcgggtctgggaccaagctggagatcaaacgggggtggcgggtggctcgggcgggtgggtgggtggcggcggtatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaaggggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttcttaaaaaatcaacagctctgcaaaactgat  
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtgcacagtcctctctgatctggagcccaa  
 10 atcttctgacaaaactcacatccccaccgtccccagcacctgaactcctgggtggaccgtcagttctcttccccccaaaaccca  
 aggacacctcatgatctccggaccttgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaac  
 tggtagctggacggcggtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagc  
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcga  
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctgaccaagaa  
 15 ccaggtcagcctgacctgctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac  
 aactacaagaccacgcctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca  
 gcagggggaacgtcttctcatgctcctgatgcatgaggtctgcacaaccactacagcagaagagcctctccctgtctccgggtaa  
 agcggatccttcgaacatggagaacaacaaaacctcagtggttcaaaatccattaataattttgaagtaaagaccatacatgggagc  
 aagtcagtgactctgggatctatctggacagtagttacaaaatggattatcctgaaatgggcatatgcataataataataaagaact  
 20 tccataagagcactggaatgtcatctcgtctggtacggatgtggacgcagccaacctcagagagacattcatgggctgaaataacc  
 aagtcaggaataaaaaatgatcttactcgtgaagacattttgaattaatggatagtggttctaaggaagatcatagcaaaaggagcagct  
 ttgtgtgtgattctaagccatggtgatgaaggggtcatttatgggacaaatgggcctgttgaaactgaaaaagttgactagcttctcag  
 aggcgactactgccggagctgactggaaagccgaaactcttcattcaggcctgccgggggtacggagctggactgtggcattga  
 gacagacagtgaggactgatgaggagatggcttgcagaagataccggtggaggctgacttctgtatgcttactctacagcacctgg  
 25 ttactattcctggagaaattcaaaggacgggtcgtggttcatccagtcctttgcagcatgctgaagctgtacgcgcacaagctagaatt  
 tatgcacattctcactcgcgttaacaggaaggtggcaacggaattcagtccttctccctggactccactttccacgcaagaaacaga  
 tcccggtgattgtgtccatgctcacgaaagaactgtactttatcactagctcgagatcgatg

Amino acid sequence;

30 mdfqvqifsflisavimsrgvdivltqspttiaaspgekvititrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
 ssgtsyslaintmetedaatyccqwsstpltfsgstkleikrgggsgggsgggsgqvlkeagplvqptqlslctvsgfs  
 ltsdgvhwirppgkglewmgiiydgtdynsaiksrlsirdtsksqvlkinslqtdtdamyycaarihfdywggqvmvtv  
 ssdlepkssdkhtspspapellggpsvflfppkpkdltmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree  
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrdeltknqvsitclvkgfypsdiav  
 35 ewesngqpennykttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgkadpsnmennktsvd  
 sksinnfevktihgksvdsgiyldssykmndypemgiciiiinnknfhkstgmssrsgtdvdaanlretfmglkyqvrnkndltr  
 edilelmdsvskedhskrssfcvvlshgdegviygtngpvelkkltstffrgdyrsltgkpklfiiqacrgtelcdgietsdgtdee  
 macqkipveadflyaystapgyyswrnskdgswfqslscmlklyahklefmhiltrvnrkvatefesfslstdthakkqipiciv  
 smltkelyfyh

40

### 137.1D8 scFv hIgG1 (SSS-P)H P238S CH2 WCH3-mcasp3-TM/CT

Nucleotide sequence:

Aagcttatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgttaagttacatgtactggtag  
 45 cagcagaagtcaggcgccctcccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacccatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc

tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatctc  
 aggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagaccctgtccctcatgactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttcttaaaaaatcaacagtctgcaaactgat  
 5 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctga

Amino acid sequence:

mdfvqvifsfllisasvmsrgvdivltqspttiaaspgekvititrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
 sgsgtsyslaintmetedaatyycqqsstpltfsggtkleikrgggsgggsgggsgqvlkeagpqlvqptqlstlctvsgfs  
 10 ltsdgvhwirppgkglewmgiyydgtdynsaiksrlsisrdtsksqvlkinslqddtamyycaihfdywgqgvmvttv  
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmistrpevtcvvvdvshedpevkfnwyvdgvevhnaktkpree  
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsltclvkgfypsdiav  
 ewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispgakdpsnsnmennkts  
 vdsksinnfevktihgsksvdsgiyldssykmdypemgiciniinnknfhkstmssrsrgtdvdanlretfmgikyqvrnknd  
 15 ltredilelmdsvskedhskrssfvcvilshgdegviygtngpvelkkltstffrgdyrcsltgkpklfiiacrgteldcgietdsgtde  
 emacqkipveadflyaystapgyyswrnskdgswwfiqlscmlklyahklefmhiltrvnrkvatefesfslstdfhakkqipei  
 vsmlltkelyfyh

### 138.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-mcasp8-TM/CT

20 Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgtaagttacatgtactgtgtac  
 cagcagaagtcaggcgctcccctaaactctggattatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccatttactgtcagcagtgtagtagtactccgc  
 25 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatctc  
 aggtgcagctgaaggaggcaggacctggcctgggtgcaaccgacacagaccctgtccctcatgactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagttttcttaaaaaatcaacagtctgcaaactgat  
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcaggtcacagtctcctctgatctggagcccaa  
 30 atcttctgacaaaactcacatccccaccgtccccagcacctgaactcctgggtggaccgtcagtttctcttccccccaaaaccca  
 aggacacctcatgatctcccgaccctgaggtcacatgcgtgggtggagcgtgagccacgaagacctgaggtcaagttcaac  
 tggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtgtgcagc  
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagccccatcga  
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccatccgggatgagctgaccaagaa  
 35 ccaggtcagcctgacctgctggtcaaaaggtctatccagcgacatcgccgtggagtgaggagagcaatgggcagccgggagaac  
 aactacaagaccacgcctcccgtgctggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca  
 gcaggggaacgtcttctcatgctccgtgatgcagtgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa  
 agcggatccttgaacatggatttcagagttgtctttatgctattgctgaagaactgggcagtgagacctggctgcctcaagttcct  
 gtgcttggactacatcccacacaagaagcaggagaccatcgaggatgccagaagctatttctgagggtcggggaaaaggggatgt  
 40 tggaggaaggcaatctgtcttctgaaagagctgctttccacatcagtcgggtgggacctgctggtaacttcttagactgcaaccga  
 gaggagatggtgagagagctgcgggatccagacaatgccagatttctcctacagggtcatgtctttaagctctcagaagaagtg  
 agcgagttggaattgagatctttaagttcctttgaacaatgagatcccaaatgtaagctggaagatgacttgagcctgcttgaaat  
 tgtgaaatggagaagaggacatgctggcagaaaataacttgaaaccctaaaaatcaatctgtgaccaggtcaacaagagcctgct  
 ggggaagatcgaggattatgaaagatcaagcacagagagaagaatgagcctgaaggaagggaagagttgccaccttcagtttgg  
 45 atgagatgagcctcaaaatggcggaactgtgtgactgccaagagaacaagacagtgagtcacggacttcagacaaagtttaccaa  
 atgaagaacaaacctggggatactgtctgatcatcaacaatcatgatttcagcaaggccgggaagacataacccaactccgaaaa



atgaaggacagaaaaggaacagactgtgataaagaggctctgagtaagacctttaaggagcttcattttgagatagtatcttacgacg  
actgcactgcaaatgaaatccacgagattctagaaggctaccaagcgcagaccacaagaacaaagactgcttcatctgctgtatcct  
atcccacgggtgacaaggggtgctgtctatggaacggatgggaaggaggcctccatctatgacctgacatcttacttcactggttcaagt  
gccccttccctgtctgggaaacccaagatcttttcttattcaggcttgccaaggaagtaactccagaaaggagtgcctgatgaggcagg  
5 cttcagacgaacagaaccacactttagaagtggattcatcatctcacaagaactatattccggatgaggcagactttctgctgggaatgg  
ctacgggtgaagaactgcgttctaccgagatcctgtgaatggaacctggatatattcagtcactttgccagagcctgagggaaagatgt  
cctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacaggaggaacaagggaaagca  
gatgccacagcccaccttcacactacggaagaagctcttctccctccctaatactgagatcgatt

10 Amino acid sequence

mdfqvqifsflisavimsrgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
sgsgtsyslaintmetedaatyycqqwsstpltfsgstkleikrgggsgggsgggsgggsgvqlkeagpglvqptqtlstctvsfgs  
ltsdgvhwirppgkglewmgiiyydggt dynsaiksrlsisrdsksqvfkinslqtdtdamyycarihfdywgqgvmv  
ssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpre  
15 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsltclvkgyfypsdiav  
ewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhcalnhhtqkslslspgkadsnmdfqsclyai  
aeelgsedlaalkflclidyphkkqetiedaqklflrlrekgmleegnlsflkellfhisrwdllvnfldcnreemvrelrdpdnaqis  
pyrvmlfklseevselelrsfkflnneipkckleddlsleifvemekrtmlaennletlksicdqnksllgkiedyerssterrms  
legreelpsvldemslkmaelcdspreqdsesrtsdkvyqmknkprgycliinnhdfskareditqlrkmkdrkgtdcdkeal  
20 sktfkelhfeivsyddctaneiheilegyqsadhknkdcficcilshgdkgvvygtdgkeasiydltsyftgskcpslsgkpkiffi  
qacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvkncvsyrdpvngtwiyqslcqlsrercpqgddil  
siltgvnydvsnkddrmkgkqmpqptflrklkffpp

**139.1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-mcasp8-TM/CT**

25 Nucleotide sequence

aagcttatggattttcaagtgcagattttcagcttctgctaatactgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtac  
cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
ggctctgggacctcttattctctcgcaatcaacacctggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
30 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtcgggtggcggcggtatc  
aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgactgtctctgggttctcattaa  
ccagcgatggtgtacactggattcgacagcctccaggaaaggggtctggaatggatgggaataatatattatgatggaggcacagatt  
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gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa  
35 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggatcgtcagtcctctctccccccaaaacca  
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gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcga  
gaaaacatctccaaagccaagggcagccccgagaaccacaggtgtacacctgccccatcccggtatgagctgaccaagaa  
40 ccaggtcagcctgacctgctggtcaaaggtcttatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac  
aactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca  
gcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa  
agcggatcctcgaacatggattccagagttgtcttattgtattgtgaagaactgggcagtgaaagacctggctgcctcaagttcct  
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45 tggaggaaggcaatctgtcttctgaaagagctgctttccacatcagtcgggtgggacctgctgggtcaacttcttagactgcaaccga  
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 ggggaagatcgaggattatgaaagatcaagcacagagagaagaatgagccttgaaggagggaagagttgccaccttcagtttgg  
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 5 atgaagaacaaacctcggggatactgtctgatcatcaacaatcatgatttcagcaaggcccggaagacataaccaactccgaaa  
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 atccacgggtgacaagggtgtcgtctatggaacggatgggaaggaggcctccatctatgacctgacatcttacttactggttcaaagt  
 gcccttcctgtctgggaaaccaagatcttttcattcaggcttgccaaggaaagtaactccagaaaggagtgccatgatgaggcagg  
 10 cttcagacagaaccacacttagaagtggattcatctcacagaactatattccgatgaggcagactttctgctgggaatgg  
 ctacggatgaagaactgcgtttcctaccgagatcctgtgaatggaacctggtatattcagtcactttgccagagcctgagggaaagatgt  
 cctcaaggagatgacattcttagcatcctgactggcgtgaactatgacgtgagcaataaagacgacaggagggaacaagggaagca  
 gatgccacagcccaccttcacactacggaagaagctcttctccctccctaatgactcgagatcgattc

15 Amino acid sequence

mdfqvqifsflisavimsrgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
 sgsgtsyslaintmetedaatyccqwsstpltfsgtkleikrgggsgggsgggsgggsgvqlkeagpglvqptqtlsltctvsgfs  
 ltsdgvhwirppgkglewmgiyydggt dynsaiksrlsirdtsksqvfkinslqtdtdamyycaihfdywggvmvtv  
 ssdlepkssdkthtspspapellggssvflfpkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpree  
 20 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltclvkgyfypsdiav  
 ewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslslspgkadsnmdfqsclyai  
 aeelgsedlaalkflcldyiphkkqetiedaqklflrrekgmleegnlsfklfhisrwdllvnfldcnreemvrelrdpdnaqis  
 pyrvmlfklseevselelrsfklfnneipkckleddlsleifvemekrtmlaennletlksicdqnksllgkiedyerssterms  
 legreelppsvldemslkmaelcdspreqdsesrtdskvyqmknkprgycliinnhdfskareditqlrkmkdrkgtdcdkeal  
 25 sktfkelhfeivsyddctaneiheilegyqsadhknkdcfcicilshgdkgvvygtdgkeasiydltsyftgskcpslsgkpkiffi  
 qacqgsnfqkgvpdeagfeqqnhtlevdssshknyipdeadflgmatvknvsvrpdvngtwiyqlscqlsrercpqqddil  
 siltgvnydvsnkddrrnkqkmpqptflrklkffpp

140.1D8 scFv hIgG1 (SSS-P)H WCH2 WCH3-hcasp3-TM/CT

30 aagcttatggattttcaagtgcagatttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactgtgac  
 cagcagaagtcaggcgctccctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
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 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtggcgggcgatctc  
 35 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagctctgcaaactgat  
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa  
 atcttctgacaaaactcacacatccccaccgtccccagcactgaactcctgggtggaccgtcagttctcttcccccccaaaacca  
 40 aggacacctcatgatctcccggaccctgaggtcagatgcgtggtggtagctgagccacgaagacctgaggtcaagttcaac  
 tggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagacaacagcacgtaccgtgtggtcagc  
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcga  
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 ccaggtcagcctgacctgctggtcaaaggctctatccagcgacatcgccgtggagtgggagagcaatgggcagccggagaac  
 45 aactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca  
 cgagggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaa

agcggatccttgaacatggagaacactgaaaactcagtgattcaaaatccataaaaatttgaaccaagatcatacatggaagc  
 gaatcaatggactctggaatatccctggacaacagttataaaatggattatcctgagatgggtttatgtataataattaataagaatttt  
 cataaaagcactggaatgacatctcggctcgtgtacagatgtcgcagcgaacctcagggaaacattcagaaactgaaatatgaag  
 tcaggaataaaaatgatcttacacgtgaagaaattgtggaattgatgcgtgatgtttctaaagaagatcacagcaaaaggagcagtttg  
 5 tttgtgtgcttctgagccatggggaagaaggaataattttggaacaaatggacctgttgacctgaaaaaataacaaactttttcagagg  
 ggatcgtttagaagtctaactgaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattgagacag  
 acagtgggtgtgatgatgacatggcgtgtcataaaataaccagtggaggccgacttctgtatgcatactccacagcacctggttattattc  
 ttggcgaaattcaaaggatggctcctggttcacagtcgctttgtccatgctgaaacagtatgccgacaagctgaatttatgcacatt  
 cttacccgggttaaccgaaaggtggcaacagaatttgagtccttttcctttgacgctacttttcatgcaaaagaaacagattccatgtattgt  
 10 ttcattgctcaciaaagaactctattttatcactaactcagatcgata

# Amino acid sequence:

mdfqvqifsfllisasvmsrgvdivltqspttiaaspgekvtitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
 sgshtsyslaintmetedaatyccqqsstpltfsgstkleikrgggsgggsgggsgqvlkeagpglvqptqlsltctvsgfs  
 15 ltsdgvhwirpppgklewmgiyydggttdynsaiksrlsirdtsksqvfkinslqtdtdamyyarihfdywgqgvmvtv  
 ssdlepkssdkthtspspapellggpsvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpree  
 qynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsiltclvkgfypsdiav  
 ewesngqpennykthppvldsdsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslslspgkadsnmentensvd  
 sksiknlepkiihgsemdsgisldnsykmdypemglciinnknfhkstgmtsrsgtdvdaanlretfmlkyevmknldtre  
 20 eivelmrdvskedhskrssfvcvllshgeegiifgtngpvdllkitnffrgdrclstgkpklfiiqacrgtelcgiestdsgvdddm  
 achkipveadflyaystapgyyswrnskdgswfqslcamlkqyadklefmhiltrvnrkvatefesfsdatfhakkqipcivs  
 mltkelyfyh

## 141. 1D8 scFv hIgG1 (SSS-P)H P238SCH2 WCH3-hcasp3-TM/CT

25 Nucleotide sequence:  
 aagcttatggattttcaagtgcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgtcactca  
 gtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtagc  
 cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgaggtagtactccgc  
 30 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcggcggtgggtgggtcgggtggcgccgatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcacatgcactgtctctgggttctcattaa  
 ccagcgatgggtgtacactggattcgacagcctccaggaaagggcttggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaaatcaacagctgcaaaactgat  
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa  
 35 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggatcgtcagtccttcttccccccaaaaccca  
 aggacacctcatgatctcccgaccctgaggtcacatgcgtggtgggtggacgtgagccacgaagacctgaggtcaagttcaac  
 tggtagtggtgacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtaaacagcacgtaccgtgtggtcagc  
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtcaaggtctccaacaaagccctccagcccccatcga  
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgcccccatccgggatgagctgaccaagaa  
 40 ccaggtcagcctgacctgcctgggtcaaaggctctatccagcgacatcgccgtggagtgaggagcaatgggcagccgggagaac  
 aactacaagaccacgctccctgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca  
 gcaggggaaagcttctctatgctccgtgatgaggtctgcacaaccactacacgcagaagagcctctcctctgctccgggttaa  
 agcggatccttgaacatggagaacactgaaaactcagtgattcaaaatccataaaaatttgaaccaagatcatacatggaagc  
 gaatcaatggactctggaatatccctggacaacagttataaaatggattatcctgagatgggtttatgtataataattaataagaatttt  
 45 cataaaagcactggaatgacatctcggctcgtgtacagatgtcgcagcgaacctcagggaaacattcagaaactgaaatatgaag  
 tcaggaataaaaatgatcttacacgtgaagaaattgtggaattgatgcgtgatgtttctaaagaagatcacagcaaaaggagcagtttg

5 tttgtgtccttctgagccatggtgaagaaggaataatTTTTGGAACAAATGGACCTGTTGACCTGAAAAAATAACAACTTTTCAGAGG  
 ggatcgtttagaagtctaactggaaaacccaaacttttcattattcaggcctgccgtggtacagaactggactgtggcattgagacag  
 acagtgggtgtgatgatgacatggcgtgtcataaaataaccagtggaggccgacttctgtatgcatactccacagcacctgggtattattc  
 ttggcgaaattcaaaggatggctcctggttcatccagtcgctttgtgcatgctgaaacagtatgccgacaagcttgaatttatgcacatt  
 cttacccgggttaaccgaaaggtggcaacagaatttgagtccttttcttgacgctacttttcatgcaaagaaacagattccatgtattgt  
 ttcatgctcacaaaagaactctattttatcactaactcgagatcgataa

Amino acid sequence:

10 mdfqvqifsflisavimsrgvdivltqspttiaaspgekvitcrasssvsymywyqqksgasplwiydtsklasgvpnrfsg  
 ssgtsyslaintmetedaatyccqqsstpltfsgstkleikrgggsgggsgggsggsqvqlkeagpglvqptqlslctvsgfs  
 ltsdgvhwirpppgklewmgiiyydgtdynsaiksrslsrdtsksqvlkinslqtdtdamyycaihfdywggqvmvttv  
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpre  
 qynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvslltclvkgfypsdiav  
 ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhhealhnhytqkslspsgkadsnmentensvd  
 15 sksiknlepkiihgsesmdsgisldnsykmdypemglciinnknfhkstgmtsrsgtdvdaanlretfnlkyevrnkndltre  
 eivelmrdvskedhskrssfvcvllshgeegiifgtngpvdllkitnffrgdrsrsltgkpklfiiqacrgtelcgiestdsgvdddm  
 achkipveadflyaystapgyyswrnskdgswfiqslcamlkqyadklefmhiltrvrnkvatfefesfsdatfhakkqipcivs  
 mltkelyfyh

20

#### 142.1D8 scFv hIgG1 (SSS-S)H WCH2 WCH3-hcasp8-TM/CT

Nucleotide sequence:

25 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 gtctccaacaaccatagctgcatctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtac  
 cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagttccaaatcgcttcagtggcagtg  
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccacttattactgtcagcagtgagtagtactccgc  
 tcacgttcgggtctgggaccaagctggagatcaaacggggtggcgggtggctcgggcgggtgggtgggtggcgccggatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagaccctgtccctcacatgcactgtctctgggttctcattaa  
 ccagcgatggtgtacactggattcgacagcctccaggaaagggctggaatggatgggaataatatattatgatggaggcacagatt  
 30 ataattcagcaattaaatccagactgagcatcagcaggacacctccaagagccaagtttcttaaaaaataacagctgcaaactgat  
 gacacagccatgtattactgtccagaatccactttgattactggggccaaggagtcaggtcacagtcctctgtatctggagcccaa  
 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggaccgtcagttctctctccccccaaaaccca  
 aggacaccctcatgatctcccggaccctgaggtcatatgcgtggtgggtggacgtgagccacgaagaccctgagggtcaagttcaac  
 tggtagctggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtagaacagcacgtaccgtgtggtcagc  
 35 gtctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctccagcccccatcga  
 gaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccggtatgagctgaccaagaa  
 ccaggtcagcctgacctgctgtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccgggagaac  
 aactacaagaccacgctcccgctgctggactccgacggctccttctctacagcaagctcaccgtggacaagagcaggtggca  
 gcagggggaacgtcttctatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctcctgtctccgggtaa  
 40 agcggatccttgaacatggacttcagcagaaatctttatgatattggggaacaactggacagtgaagatctggcctccctcaagttcc  
 tgagcctggactacattccgaaaggaagcaagaacctatcaaggatgccttgatgtattccagagactccaggaaaagagaatgt  
 tggaggaaagcaatctgtccttctgaaggagctgcttccgaattaatagactggatttctgattacctacctaactagaaagg  
 aggagatggaaaggggaacttcagacaccaggcagggtcctcaattctgcctacagggtcatgctctatcagatttcagaagaagtga  
 gcagatcagaattgaggtctttaagttcttttgaagaggaaatctccaaatgcaaactggatgatgacatgaacctgctggatatttc  
 45 atagagatggagaagagggtcatcctgggagaaggaaagtggacatcctgaaaagagtcgtgtcccaaatcaacaagagcctgct  
 gaagataatcaacgactatgaagaattcagcaaaagagagaagcagcagcctgaagggaagtcctgatgaattttcaaatggggagg

agttgtgtgggtaatgacaatctcggactctccaagagaacaggatagtgatcacagactttggacaaagtttaccaaatgaaaag  
 caaacctcggggatactgtctgatcatcaacaatcacaattttgcaaaagcacgggagaaagtgccaaacttcacagcattagga  
 caggaatggaacacacttgatgcaggggctttgaccacgacctttgaagagcttcattttgagatcaagccccacgatgactgcaca  
 gtagagcaaactatgagattttgaaaatctaccaactcatggaccacagtaacatggactgcttcactgctgtatcctctcccatggag  
 5 acaagggcatcatctatggcactgatggacaggagggcccccactatgagctgacatctcagttcactggtttgaagtgcccttcctt  
 gctggaaaacccaaagtgtttttattcaggcttgcagggggataactaccagaaaggtatacctgttgagactgattcagaggagca  
 accctatttagaaatggatttatcatcacctcaaacgagatatatcccgatgaggctgactttctgctggggatggccactgtgaataa  
 ctgtgtttcctaccgaaacctgcagagggaacctggtacatccagtcactttgccagagcctgagagagcgatgtcctcaggcgga  
 tgatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagcc  
 10 tactttcacactaagaaaaaactgtcttcccttctgattgagcatgcatcgata

# Amino acid sequence:

mdfqvqifsfllisasvmsrgvdivltqspttiaaspgekvitcrasssvsmywyqqksgasplwiydtsklasgvpnrfsg  
 15 ssgtsyslaintmetedaatyccqwsstpltfsgtkleikrgggsgggsgggsgvqlkeagpglvqptqtlstctvsfsgf  
 ltsdgvhwirpppgklewmgiiyydggtynsaiksrlsirdtsksqvfkinslqtdtdamyyarihfdywgqgmvtv  
 ssdlepkssdkthtspspapellggsplfpkpkdltmisrtpevtcvvdvshedpevkfnwyvdgvevhnaktkpree  
 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvylppsrdeltknqvsltclvkgyfypsdiav  
 ewesngqpennykttppvldsdsfflyskltvdksrwqqgnvfscsvmhleahnytkslslspgkadsnmdfsrnllydi  
 20 geqldsedlaslkflsldyipqrkqepikdalmlfqlrlekrmlselsfkelldfrinrldlitylnrkeemerelqtpraqisay  
 rvmlyqiseevsrslrsfkflqeeiskckldddmnlldifiemekrvilgegkldilkrvcaqinkslkiindyefskerssle  
 gspdefsngeelcgvmtisdspreqdsesqtdkvyqmkskprgycliinnhnfakarekvpklhsirdnrgthldagaltttfee  
 lhfeikphddctveqiyelkiyqlmdhsnmdcficilshgdkgiiygdgqeapiyeltsqftglkcpslagkpkvffiqacqg  
 dnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvsvrnpaegtwyiqslcqlsrercprgddiltitvevny  
 25 evsnkddkknmgkqmpqptflrkklvfspd

## 143.1D8 scFv hIgG1 (SSS-S)H P238SCH2 WCH3-hcasp8-TM/CT

### Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
 30 gtctccaacaaccatagctgcattctccaggggagaaggtcaccatcacctgccgtgccagctccagtgaagtacatgtactggtag  
 cagcagaagtcaggcgctccctaaactctggatttatgacacatcaagctggcttctggagttccaaatcgcttcagtggcagt  
 ggtctgggacctcttattctctcgcaatcaacacatggagactgaagatgctgccatttactgtcagcagtgaggtagtactccgc  
 tcacgttcgggtctgggaccaagctggagatcaaacgggtggcgggtggctcggcggtggtgggtcgggtggcgcgatctc  
 aggtgcagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctcatgactgtctctgggttctcattaa  
 35 ccagcgatggtgtacactggattcagacgctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
 ataattcagcaattaaatccagactgagcatcagcagggacacctccaagagccaagttttcttaaaaaatcaacagtctgcaaaactgat  
 gacacagccatgtattactgtgccagaatccactttgattactggggccaaggagtcagtggtcacagtctcctctgatctggagcccaa  
 atcttctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggtggatcgtcagttcctcttccccccaaaaccca  
 aggacacctcatgatctcccgaccctgaggtcatatgcgtggtggtggacgtgagccacgaagacctgaggtcaagttcaac  
 40 tggtagctggcagcgctggaggtgcataatgccaaagacaaagccgctgggagagcagtagaacagcacgtaccgtgtggtcagc  
 gtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctcaacaagccctccagccccatcga  
 gaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatcccggtatgagctgaccaagaa  
 ccaggtcagcctgacctgcctggtcaaaggcttctatccagcgacatcgccgtggagtgggagagcaatggcgagccgggagaac  
 aactacaagaccacgctcccgtgctggactccgacggctccttctctctacagcaagctcaccgtggacaagagcaggtggca  
 45 gcaggggaacgtcttctcatgctccgtgatgcatgaggtctgcacaacctacacgcagaagagcctctccctgtctccgggtaa  
 agcggatccttgaacatggacttcagcagaaatcttatgatattgggaacaactggacagtgaagatctggcctccctcaagttcc

5 tgagcctggactacattccgcaaaggaagcaagaacccatcaaggatgccttgatgtattccagagactccaggaaaagagaatgt  
 tggaggaaaagcaatctgtccttcctgaaggagctgctctccgaattaatagactggatttgctgattacactacaaactagaaagg  
 aggagatggaaaggggaacttcagacaccaggcagggtcaaattctgcctacagggtcatgctctatcagatttcagaagaagtga  
 gcagatcagaattgaggtcttttaagtttctttgcaagaggaaatccaaatgaaaactggatgatgacatgaacctgctggatatttc  
 10 atagagatggagaagagggtcatcctgggagaaggaaagtggacatcctgaaaagagtctgtgccaaatcaacaagagcctgtg  
 gaagataatcaacgactatgaagaattcagcaaagagagaagcagcagcctgaaggaaagtcctgatgaattttcaaatggggagg  
 agttgtgtggggaatgacaatctcggactctccaagagaacaggatagtgaatcacagactttggacaaagtttaccaaatgaaaag  
 caaacctcggggatactgtctgatcatcaacaatcacaattttgcaaaagcacgggagaaagtgccaaacttcacagcattaggga  
 caggaatggaacacacttgatgcaggggctttgaccacgacctttgaagagcttcattttgagatcaagccccacgatgactgcaca  
 15 gtagagcaaactctatgagattttgaaaatctaccaactcatggaccacagtaacatggactgcttcactgctgtatcctctccatggag  
 acaagggcacatctatggcactgatggacaggagcccccatctatgagctgacatctcagttcactggttgaaagtgccctccctt  
 gctggaaaacccaaagtgtttttattcaggctgtcagggggataactaccagaaaaggtatacctgttgagactgattcagaggagca  
 accctatttagaaatggatttatcatcacctcaaacgagatatatccggatgaggctgactttctgctggggatggccactgtgaataa  
 ctgtgttctaccgaaacctgcagagggaacctggtacatccagtcactttgccagagcctgagagagcagatgtcctcaggcga  
 20 tgatattctaccatcctgactgaagtgaactatgaagtaagcaacaaggatgacaagaaaaacatggggaaacagatgcctcagcc  
 tactttcacactaagaaaaaactgtcttccctctgattgagcatgcatcgataa

Amino acid sequence:

20 mdfqvqifsfllisasvimsgvdivltqspttiaaspgekvtitcrassvsymywyqqksgasplwiydtsklasgvpnrfsg  
 sgshtsyslaintmetedaatyycqwsstpltfsgstkleikrgggsgggsgggsgvqlkeagpglvqptqtlstctvsgfs  
 ltsdgvhwirpppgklewmgiiyydgtdynsaiksrlsrdtsksqvlkinslqtdtdamyycaihfdywgqgvmvtv  
 ssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtevtcvvvdvshedpevkfnwyvdgvevhnaktkpre  
 25 qynstyrvsvltvlhqdwlngkeykckvsnkalpapietiskakgpprepqvylppsrdeltknqvslclvkgyfypsdiav  
 ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhytqkslslspgkadsnmdfsmlydi  
 geqlsdslaskflsldyipqrkqepikdalmlfqlrlekrmlleenslflkellfrinrldlitylntrkeemerelqtpraqisay  
 rvmlyqiseevsrslrsfklflqeeiskckldddmnlldifiemekrvilgeglklilkrvcaqinkslkiindyefskerssle  
 gspdefsngeelcgvmtisdspreqdsesqldkvyqmkskprgycliinnhnfakarekvpklhsirdrnghldagalttfee  
 30 lhfeikphddctveqiyeilkiyqlmdhsnmdecficclshgdkgiiygtgdqeapiyeltsqftglkcpslagkpkvffiqacqg  
 dnyqkgipvetdseeqpylemdlsspqtryipdeadflgmatvnnvsvrnpaegtwyiqslcqlsrercprgddilttevnv  
 evsnkddkknmgkmpqptflrklvfpd

144.

### 35 145. hCTLA4 IgAH IgACH2CH3

Nucleotide sequence:

atggcttgcccttgatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgactctcctgtttttcttcttcat  
 ccctgtcttctgcaaagcaatgcacgtggccagcctgctgtgtgactggccagcagccaggcatcggcagcttctgtgtgagtat  
 40 gcatctccaggcaaaagccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcgggcaacct  
 acatgacgggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctactatccaagg  
 actgagggccatggacacgggactctacatctgcaaggtggagctcatgtaccaccgccatactacctgggcataggcaacggaa  
 ccagatttatgtaattgatccagaacctgtcccagattctgatcagccagttccctcaactccactaccccatctccctcaactccac  
 ctaccccatctccctcatgtgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgtcct  
 cacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgtgttcaaggac  
 45 cacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagacctca  
 cttgcatgctgcctaccccgagtccaagaccccgctaaccgccacctctcaaaatccggaaacacattccggcccgaggtccac

ctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatg  
 tgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacgtgactgggcatcccgaggagccagccagg  
 gcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggg  
 ccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgtgtcat  
 5 ggcggagggtgacggcacctgctactgataatctaga

Amino acid sequence:

maclgfqrhkaqlnlaartwpctllffllfipvfckamhvaqpavvlassrgiasfvceyaspkgatevrvtlrqadsqvteveca  
 tymtgneltflddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpdsdqpvpstptps  
 10 pstptpspscchprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrdlcgysvssvlpgaepwnhg  
 ktftctaaypesktpatlsksngntfrpevhllpppseelalnelvltclargfspkdvlvrlqgsqelprekyltwasrqepsqg  
 ttfavtsilrvaaedwkkgdtdfscmvghealplafktidrlagkpthvnsvvmaevdgtcy

#### 146. hCTLA4 IgA WH WCH2 T4CH3 (hCTLA4 IgAH IgACH2CH3)

15 Nucleotide sequence:

atggcttgccttggatttcagcggcacaaggctcagctgaacctggctgccaggacctggccctgactctcctgtttttcttcttcat  
 ccctgtcttctgcaaagcaatgcacgtggccagcctgctgtggtactggccagcagccgaggcatcggcagcttctgtgtgagtat  
 gcatctccaggcaaagccactgaggtccgggtgacagtgttcggcaggctgacagccaggtgactgaagtctgtcggcaacct  
 acatgacggggaatgagttgaccttctagatgattccatctgcacgggcacctccagtggaaatcaagtgaacctcactatccaagg  
 20 actgagggccatggacacgggactctacatctgcaagggtggagctcatgtaccaccgccatactacctgggcataggcaacggaa  
 ccagatttatgtaattgatccagaacctgccagattctgatcagccagttccctcaactccacctaccccatctccctcaactccac  
 ctaccccatctccctcatgctgccacccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcct  
 cacgtgcacactgaccggcctgagagatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggac  
 cacctgaccgtgacctctgtggctgctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttca  
 25 ctgcaactgctgcctaccccgagtcgaagaccccgctaaccgccacctctcaaaatccggaaacacattccggcccgaggtccac  
 ctgctgccgccgccgtcggaggagctggccctgaacgagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatg  
 tgctggttcgctggctgcaggggtcacaggagctgccccgcgagaagtacgtgactgggcatcccgaggagccagccagg  
 gcaccaccaccttcgctgtgaccagcatactgcgcgtggcagccgaggactggaagaagggggacaccttctcctgcatggtggg  
 ccacgaggccctgccgctggccttcacacagaagaccatcgaccgcttggcgggtaaaccacccatgtcaatgtgtctgtgtcat  
 30 ggcggagggtgactgataatctaga

Amino acid sequence:

Maclgfqrhkaqlnlaartwpctllffllfipvfckamhvaqpavvlassrgiasfvceyaspkgatevrvtlrqadsqvteveca  
 atymtgneltflddsictgtssgnqvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpdsdqpvpstptp  
 35 spstptpspscchprlsihrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrdlcgysvssvlpgaepwnh  
 gktftctaaypesktpatlsksngntfrpevhllpppseelalnelvltclargfspkdvlvrlqgsqelprekyltwasrqepsq  
 gtttfavtsilrvaaedwkkgdtdfscmvghealplafktidrlagkpthvnsvvmaevd

#### 147. hIGA WH WCH2 T18CH3

40 Nucleotide sequence:

Tgatcagccagttccctcaactccacctaccccatctccctcaactccacctaccccatctccctcatgctgccacccccgactgtcac  
 tgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgagagatgcctcaggt  
 gtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgacctgtggtgctacagcgtgtcc  
 agtgcctgccgggctgtgccgagccatggaacctgggaagaccttcaactgactgtgcctaccccgagtcgaagaccccgct  
 45 aaccgccacccttcaaaatccggaacacattccggcccgaggtccacctgctgccgccgccgtcggaggagctggccctgaac  
 gagctggtgacgctgacgtgcctggcacgtggcttcagccccaaggatgtgctggttcgctggctgcaggggtcacaggagctgc

cccgcgagaagtacgtgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccagcatactgcgcgt  
ggcagccgaggactggaagaagggggacaccttctcgtcatggtgggccacgaggccctgccgctggccttcacacagaagac  
catcgaccgcttggcgggtaaatgataatctaga

5 Amino acid sequence:

dqvpvstptpsptpspspcchprlslhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgppdrdlcgcysvssv  
lpgcaepwnhgktfictaaypesktpatlsksngtfrpevhllpppseelalnelvtltclargfspkdvlvrwlqgsqelprekyl  
twasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghealplafqtktidrlagk

10 148. 2h7 scFv IgA WH WCH2 T4CH3

Nucleotide sequence:

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgcccacccaacctggcttctggagtcctgctgcttcagtgga  
15 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc  
tctcaggttatctacagcagcttggggctgagctggtagggcctggggcctcagtgaaagatgctcctgcaaggcttctggctacacat  
ttaccagttacaatatgcactgggtaaagcagacacctagacagggcctggaatggattggagctattatccaggaaatggtgatact  
tctacaatcagaagtcaagggaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
20 atctgaagactctgcggctctatttctgtgcaagagtgggtactatagtaacttctactggtacttcgatgtctggggcacagggaccac  
ggtcaccgtctcttctgatcagccagttccctcaactccacctacccatctccctcaactccacctacccatctccctcatgtgccac  
ccccgactgtcactgcaccgaccggccctcaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag  
agatgcctcaggtgtcaccttcacctggacgccctcaagtgggaagagcgctgttcaaggaccacctgaccgtgacctctgtggctg  
ctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcacttgactgtgctaccccgagtc  
25 caagaccccgtcaaccgccaccctctcaaaatccggaacacattccggcccaggtccacctgctgccgccgccgtcggaggag  
ctggccctgaacgagctggtgacgtgacgtgcctggcacgtggcttcagccccaaggatgtgtggtcgtggctgcaggggtc  
acaggagctgccccgcgagaagtacgtgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag  
catactgcgcgtggcagccgaggactggaagaagggggacaccttctcgtcatggtggggccacgaggccctgccgctggccttc  
acacagaagaccatcgaccgttggcgggtaaacccacccatgtcaatgtgtctgttgcattggcggagggtgactgataatctaga

30

Amino acid sequence:

mdfqvqifslilasviiargqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgsspkpwiypasnlasgvparfsg  
sgsgtsysltisrveadaatyycqqsfnpptfgagtkleldggsgggsgggssqaylqqsgaelvrpgasvkmsckas  
gytftsynmhwwkqtrqglewigaiypngdtsynqkfkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy  
35 fdvwtgtttvssdqvpvstptpsptpspspcchprlslhrpaledlllgseailtctltglrdasgvtftwtpssgksavqgp  
pdrdlcgcysvssvlpcaepwnhgktfictaaypesktpatlsksngtfrpevhllpppseelalnelvtltclargfspkdvlr  
wlqgsqelprekyltwasrqepsqgtttfavtsilrvaaedwkkgdtfscmvghealplafqtktidrlagkphvnnsvvmaev  
d

40 149. 2H7 scFv IgA WH WCH2 T18CH3

Nucleotide sequence

aagcttgccgccatggattttcaagtgcagatttcagcttctgctaatactgcttcagtcataattgccagaggacaaattgttctctc  
ccagctccagcaatcctgtctgcattctccaggggagaaggtcacatgacttgaggccagctcaagtgttaagttacatgcactgg  
taccagcagaagccaggatcctcccccacccctggatttatgcccacccaacctggcttctggagtcctgctgcttcagtgga  
45 gtgggtctgggaccttactctctcacaatcagcagagtggaggctgaagatgctgccacttattactgccagcagtgagggtttaac  
ccaccacgttcgggtgctgggaccaagctggagctgaaagatggcgggtggctcgggcgggtggatctggaggaggtgggagc



tctcaggcttatctacagcagctctggggctgagctggtgaggcctggggcctcagtgaaagtgcctgcaaggcttctggctacacat  
 ttaccagttacaatatgcactgggtaaagcagacacctagacaggcctggaatggattggagctatttatccaggaaatggtgatact  
 tctacaatcagaagttcaagggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgac  
 atctgaagactctcggtctatttctgtgcaagagtgtgtactatagtaactcttactggtacttcgatgtctggggcacagggaccac  
 5 ggtcaccgtctcttctgatcagccagttccctcaactccactaccccatctccctcaactccactaccccatctccctcatgctgccac  
 ccccgactgtcactgcaccgaccggccctcgaggacctgctcttaggttcagaagcgatcctcacgtgcacactgaccggcctgag  
 agatgcctcaggtgtcacctcactggacgcctcaagtgggaagagcgctgttcaaggaccacctgacctgacctgtgtggctg  
 ctacagcgtgtccagtgtcctgccgggctgtgccgagccatggaacctgggaagaccttcactgactgctgcctaccccgagtc  
 caagaccccgtaaccgccaccctctcaaaatccggaacacattccggcccaggtccacctgctgccgccgctcggaggag  
 10 ctggccctgaacgagctggtgacgtgacgtgacctggcagctggttcagcccaaggatgtgctggttcgctggctgcaggggtc  
 acaggagctgccccgcgagaagtacctgacttgggcatcccggcaggagcccagccagggcaccaccaccttcgctgtgaccag  
 catactgcgctggcagccgaggactggaagaagggggacaccttctcctgcatggtgggcccagaggccctgccgctggccttc  
 acacagaagaccatcgaccgcttggcgggtaaatgataatctaga

15 Amino acid sequence:

mdfqvqifsfllisasviiargqivlsqspailsaspgekvtmtcrasssvsymhwyqqkpgsspkpwiypsnlasgvparfsg  
 sgshtsysltisrveadaatyccqwsfnpptfgagtkleldggsgggsgggssqaylqqsgaelvrpgasvkmsckas  
 gytfitsynmhwwkqtrprglewigaiypngdtsynqkfkgkatltvdksstaymqlssltedsavyfcarvvyysnsywy  
 fdvwgtgtvtvssdqvpstptpsptpspcchprlslhrpaledlllgseailtcltglrdasgvtftwtpssgksavqgp  
 20 pdrdlcgysvssvlpgaepwnhgktftctaaypesktpltatlsksgntfrpevhllpppseelalnelvtltclargfspkdvivr  
 wlqgsqelprekyltwasrqpsqgtttfavtsilrvaadwkkgdtfscmvghealplafktidrlagk

**150. G19-4 scFv (SSS-P)WH WCH2 WCH3-hCD80TMCT**

Nucleotide sequence:

25 aagcttatggattttcaagtgcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatccagatgacac  
 agactacatctcctgtctgcctctctgggagacagagtcaccatcagttgcagggcaagtcaggacattcgcaattatttaaactgg  
 tatcagcagaaaccagatggaactgttaaactcctgatctactacacatcaagattacactcaggagtcctcatcaaggttcagtggcag  
 tgggtctggaacagattattctctcaccattgccaacctgcaaccagaagatattgccacttacttttgccaacagggttaatacgttccg  
 tggacgttcgggtggaggcacaaactgtaaccaaacgggagctcgggtggcgggtggctcgggcgggtgggtgggtcgggtggcgg  
 30 cggatctatcgatgaggtccagctgcaacagctctggacctgaactggtgaagcctggagcttcaatgtcctgcaaggcctctggttac  
 tcattcactggctacatcgtaactggctgaagcagagccatggaaagaaccttgatggattggacttattaatccatacaaaggcttt  
 actacctacaaccagaaattcaagggaaggccacattaactgtagacaagtcacacagcctacatggagctcctcagctctg  
 acatctgaagactctgcagcttattactgtgcaagatctgggtactatggtgactcggactggtacttcgatgtctggggcgagggac  
 caggtcacctgtctctctgatctggagcccaaatctctgacaaaactcacacatccccaccgtccccagcacctgaactcctgggg  
 35 ggaccgtcagcttctcttcccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgctggtggtggac  
 gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaagccgcgggag  
 gagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtctgcaccaggactggctgaatggcaaggagtacaagtga  
 aggttccaacaaagccctcccagccccatcgagaaaacctctccaaagccaaagggcagccccgagaaccacaggtgtaca  
 ccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaggcttctatcccagcgacatcgcc  
 40 gtggagtgggagagcaatgggcagccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctcta  
 cagcaagctcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgctcctgatgatgaggtctgcacaaccact  
 acacgcagaagagcctctccctgtctccgggtaagcggatccttcgaacctgctccatcctgggccattaccttaatctcagtaaat  
 ggaattttgtgatgtgctgcctgacctactgctttgccccaaagatgcagagagagaaggaggaatgagagattgagaagggaagt  
 gtacgccctgtataaatcgat

45

Amino acid sequence:

mdfqvqifsflisasvimsrgvdiqmtqtsslsaslgdrvtiscrasqdirnylnwyqqkpdgtvkllyytsrlhsgvpsrfs  
 gsgtdysltianlqpediatyfcqqgntlpwtfgggtklvtkrelggggsgggsggggsdevqlqqsgpelvkpgasmsckas  
 gysftgyivnwlkqshgknlewiglinpykglttnqkfkkgkatltvdkssstaymellsitsedsavyycarsgyygdswyf  
 dvwgagttvtvssdlepkssdkthtspapellggpsvflfppkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgve  
 5 vhnaktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvsltel  
 vkgfypsdiavewesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslslspgkadps  
 nllpswaitlisvngifviccltycfaprerrrmerlrresvrpv

**151. 2e12 scFv (CCC-P)WH WCH2 WCH3-hCD80TM/CT**

10 Nucleotide sequence:

aagcttatggattttcaagtcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcacc  
 atctccagcttcttggctgtgtctctaggtcagagagccaccatctcctgcagagccagtgaaagtgtgaatattatgtcacaagtta  
 atgcagtggtaccaacagaaaccaggacagccaccaaaactcctcatctgtgcacccaacgtagaatctggggtcctgccagg  
 ttttagtggcagtggtctgggacagacttcagcctcaacatccatcctgtggaggaggatgatattgcaatgtattctgtcagcaaagt  
 15 aggaaggttccttggacgttcggtggaggcaccaagctggaaatcaaacggggtggcgggtgctcgggaggagtggtgctgggt  
 ggcgggcgatctcaggtgcagctgaaggagtcaggacctggcctggtggcgccctcacagagcctgtccatcacatgcaccgtct  
 cagggttctcattaaccggctatggtgtaaactgggtcgcagcctccaggaaagggtctggagtggtgggaatgatagggtg  
 atggaagcacagactataattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtttctaaaaatgaa  
 cagctctgaaaactgatgacacagccagataactgtgccagagatggtatagtaacttcttactatgttatggactactgggtgcaa  
 20 ggaacctcagtcaccgtctcctcagatctggagcccaaatctgtgacaaaactcacatgccaccgtgccagcacctgaactc  
 ctgggggggaccgtcagcttctcttccccccaaaacccaaggacacctcatgatctccggacccctgaggtcacatgcgtggtg  
 gtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc  
 gggaggagcagtacaacagcacgtaccgtgtgtgcagcgtcctaccgtcctgcaccaggactggctgaatggcaaggagtacaa  
 gtgcaaggctccaacaaagccctccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggt  
 25 gtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctgtcaaaggcttctatcccagcgaca  
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 30 aaagtgtacgcctgtataaatcat

Amino acid sequence:

mdfqvqifsflisasvimsrgvdivltqspaslavslgqratiscrasesveyyvtslmqwyqqkpgqppkllisaasnvesgvp  
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 35 ctvsgfsltgygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdnsksqvflkmnslqtdtdaryycardgysnfh  
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 gkadpsnllpswaitlisvngifviccltycfaprerrrmerlrresvrpv

40

**1D8 scFv (SSS-P)H P238SCH2 WCH3-hCD80TM/CT**

Nucleotide sequence:

45 aagcttatggattttcaagtcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaggagtcgacattgtgctcactca  
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cagcagaagtcaggcgcctccctaaactctggatttatgacacatccaagctggcttctggagtccaaatcgcttcagtggcagtg  
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 5 ccagcgatggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatatattatgatggaggcacagatt  
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Amino acid sequence:

20 Mdfqvqifsfllisasvimsrgvdivltspttiaaspgekvtitcrasssvsymywyqqksgasplkiwydtsklasgvpnrfsg  
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 ssdlepkssdkthtspspapellggssvflfppkpkdtlmisrtevtcvvvdvshdpevkfnwyvdgvevhnaktkpre  
 25 qynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgpprepqvytlppsrdeltknqvsitclvkgyfypsdiav  
 ewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslspsgkadsnllpswaitlis  
 vngifvicltycfaprerrmerlrresvrpv

HCD16lowFL+NL

Nucleotide sequence:

30 aagcttgccgccatgtggcagctgctcctcccaactgctctgctacttctagtttcagctggcatcgggactgaagatctccaaaggc  
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 35 cagaatggcaaggcaggaagtattttcatcataattctgacttctacattccaaaagccacactcaaagacagcggctcctacttctgc  
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 ttccacctgggtaccaagctctttctgcttggtgatgtactccttttgcagtggacacaggactatatttctctgtgaagacaaacatt  
 cgaagctcaacaagagactggaaggaccataaatttaaatggagaaaggacctcaagacaaatgacct

40 Amino acid sequence:

mwqlllptalillvsagmrtdlpkavvflepqwyrvlekdsvtlkcqgayspednstqwfhnslissqassyfidaatvddsg  
 eyrcqnlstlsdpvqlvhighwllqaprwvfkeedpihlrchswkntalhkvtylqngkgrkyfhnsdfyipkatlkdsqsyf  
 crglvgsknsvsetvnititqglavstissffppgyqvsfclvmvllfavdtglyfsvknirsstrdwkdhkfkwrkdpqdk

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended  
5 claims.

All patents, patent applications, publications, scientific articles, web sites, and other documents and materials referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced document and material is hereby incorporated by reference to the same extent as if it had  
10 been incorporated by reference in its entirety individually or set forth herein in its entirety. Additionally, all claims in this application, and all priority applications, including but not limited to original claims, are hereby incorporated in their entirety into, and form a part of, the written description of the invention. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such  
15 patents, applications, publications, scientific articles, web sites, electronically available information, and other referenced materials or documents. Applicants reserve the right to physically incorporate into any part of this document, including any part of the written description, the claims referred to above including but not limited to any original claims.

The specific methods and compositions described herein are representative of  
20 preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed  
25 herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. Thus, for example, in each instance herein, in embodiments or examples of the present invention, any of the terms “comprising”, “consisting essentially of”, and “consisting of”  
30 may be replaced with either of the other two terms in the specification. Also, the terms

“comprising”, “including”, containing”, *etc.* are to be read expansively and without limitation. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. It is also that as used herein and in the appended  
5 claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a host cell” includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the  
10 patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description  
15 and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and  
20 variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form  
25 part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art

will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

## CLAIMS

What is claimed is:

1. A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule,  
5 said binding domain polypeptide comprising a heavy chain variable region, said heavy chain variable region comprising an amino acid substitution or deletion at one or more amino acid residues; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second  
10 polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.
2. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv.
3. A protein of claim 1 wherein the one or more amino acid substitution  
15 or deletion in said heavy chain variable region is effective to increase expression or stability of said protein relative to a protein without said deletion or substitution.
4. A protein of claim 1 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.
- 20 5. A protein of claim 4 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.
6. A protein of claim 5 wherein the first target molecule and the second  
25 target molecule are different.
7. A protein of claim 5 wherein the first target molecule and the second target molecule are the same.
8. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising one or more amino acid substitution in positions 9, 10, 11, 12,  
30 108, 110, 112 in said heavy chain variable region.

9. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv comprising an amino acid substitution at position 11 in said heavy chain variable region.

5 10. A protein of claim 9 wherein the amino acid substituted for the amino acid at position 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

10 11. A protein of claim 9 wherein the amino acid substituted for the amino acid at position 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, and glutamine.

12. A protein of claim 9 where leucine is replaced by serine at position 11.

13. A protein of claim 9 where leucine is replaced by des-leucine at position 11.

15 14. A protein of claim 12 having an increased recombinant expression or stability relative to said protein not having an amino acid substitution at position 11.

15. A protein of claim 14 wherein the expression of said protein having an amino acid substitution at position 11 is 10-100 fold greater than said protein without a substitution at position 11.

20 16. A protein of claim 14 wherein said expression is in mammalian cells.

17. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.

25 18. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv and said binding domain polypeptide comprises a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

19. A protein of claim 18 wherein the amino acid at position 106 has been substituted or deleted.



20. A protein of claim 2 wherein said binding domain polypeptide binds to a tumor antigen.
21. A protein of claim 2 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.
- 5 22. A protein of claim 2 wherein said binding domain polypeptide binds to a cancer cell antigen.
23. A protein of claim 22 wherein said cancer cell antigen is a surface antigen.
24. A protein of claim 22 wherein said cancer cell antigen is an  
10 intracellular antigen.
25. A protein of claim 1 wherein said binding domain polypeptide binds to a B cell antigen.
26. A protein of claim 25 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
- 15 27. A protein of claim 2 wherein said single chain Fv binds to a B cell antigen.
28. A protein of claim 27 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.
29. A protein of claim 28 wherein said single chain Fv is selected from  
20 the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.
30. A protein of claim 2 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.
- 25 31. A protein of claim 1 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.
32. A protein of claim 31 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.
33. A protein of claim 1 wherein said binding domain polypeptide binds  
30 to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10,

CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

5                    34.     A protein of claim 1 wherein said protein is capable of forming a complex comprising two or more of said proteins.

                  35.     A protein of claim 34 wherein said complex is a dimer.

                  36.     A protein of claim 1 wherein said protein is a monomer.

                  37.     A protein of claim 1 coupled to a drug, toxin, immunomodulator,  
10 polypeptide effector, isotope, label, or effector moiety.

                  38.     A protein of claim 1 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular  
15 activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

                  39.     A protein of claim 38 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other  
20 second messengers, ion channels, and secretory pathway components.

                  40.     A protein of claim 38 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

                  41.     A protein of claim 40 wherein said induction of one or more immune  
25 effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

                  42.     A protein of claim 38 which is capable of cellular activation, wherein said activation leads to changes in cellular transcriptional activity.

                  43.     A protein of claim 42 wherein said cellular transcriptional activity is  
30 increased.

44. A protein of claim 42 wherein said cellular transcriptional activity is decreased.

45. A protein of claim 38 wherein said one or more biologically active molecules is a protease.

5 46. A protein of claim 38 wherein said one or more biologically active molecules is a cytokine.

47. A protein of claim 46 wherein said cytokine is selected from the group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

10 48. A protein of claim 38 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

49. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

15 50. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.

20 51. A protein of claim 38 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.

52. A protein of claim 1 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

25 53. A protein of claim 1 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser.

30 54. A protein of claim 53 comprising three Gly-Gly-Gly-Gly-Ser peptides.

55. A protein of claim 1 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

5 56. A protein of claim 1 wherein said binding domain polypeptide comprises a humanized immunoglobulin variable region.

57. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

10 58. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

15 59. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

20 60. A protein of claim 2 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

61. A protein of claim 1 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

25 62. A protein of claim 2 wherein said binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region.

63. A protein of claim 1 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof,

IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

64. A protein of claim 1 wherein said connecting region comprises a human IgE hinge or a portion thereof.

5 65. A protein of claim 1 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

66. A protein of claim 1 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

10 67. A protein of claim 1 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

68. A protein of claim 1 wherein said connecting region comprises a glycosylation site.

69. A protein of claim 1 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

15 70. A protein of claim 1 wherein said connecting region has one cysteine residue.

71. A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

20 72. A protein of claim 1 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

73. A protein of claim 1 where said connecting region comprises three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted and said proline residue is substituted or deleted.

25 74. A protein of claim 1 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is substituted or deleted.

75. A protein of claim 74 wherein said wild-type hinge region polypeptide is from human IgG1.

76. A non-naturally occurring single chain protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region comprising one or more amino acid deletion or substitution in positions 9, 10, 11, 12, 108, 110, 112; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

77. A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine is replaced by serine at position 11 in the first framework region of the heavy chain variable region; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

78. A protein of claim 77 further comprising a substitution or deletion of the amino acid at position 10 in the first framework region of the heavy chain variable region.

79. A protein of claim 77 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

80. A protein of claim 77 wherein the numbering of the residues is the EU index according to Kabat.

81. A protein of claim 77 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-

terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

82. A protein of claim 77 wherein said connecting region comprises an IgA hinge region or portion thereof.

5 83. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine in the connecting region, and said heavy chain constant region comprises CH2 and CH3 domains from IgG<sub>1</sub>.

10 84. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a murine IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from murine IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

15 85. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein said first, second, and third cysteine residues in the connecting region are replaced by serine and said proline residue in the connecting region is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein lysine is replaced by serine at position 322 in said CH2 region.

20 86. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein lysine is replaced by serine at position 322 in said CH2 region.

25 87. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 331 in said CH2 region.

88. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 331 in said CH2 region.

89. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein threonine is replaced by asparagine at position 256 in said CH2 region.

90. A protein of claim 81 where said single chain protein comprises a 2H7 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains from IgG<sub>1</sub> where in the CH2 domain arginine is replaced by glutamine at position 255, threonine is replaced by asparagine at position 256, proline is replaced by alanine at position 257, and glutamic acid is replaced by lysine at position 258.

91. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein lysine is replaced by glutamine at position 290 in said CH2 region.

92. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein alanine is replaced by proline at position 339 in said CH2 region.

93. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue



is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

94. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region  
5 said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

95. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region  
10 said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

96. A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region  
15 comprising CH2 and CH3 domains from IgG<sub>1</sub>.

97. A protein of claim 81 said single chain protein comprises a single chain Fv binding domain from a FC2-2 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains  
20 from IgG<sub>1</sub>.

98. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a UCHL-1 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains  
25 from IgG<sub>1</sub>.

99. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 5B9 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3  
30 domains from IgG<sub>1</sub>.

100. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

101. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises CH2 and CH3 domains from IgG<sub>1</sub>.

102. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

103. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma comprising a connecting region wherein said third cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

104. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, where in the connecting region said first cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

105. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a murine IgA hinge region, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

106. A protein of claim 82 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said connecting region comprising a human IgA hinge region and said heavy chain constant region comprises CH2 and CH3 domains from human IgA, where said CH3 comprises a deletion or substitution in  
5 four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

107. A protein of claim 81 where said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is  
10 replaced by serine, and said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

108. A protein of claim 81 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, and  
15 said heavy chain constant region comprises CH2 and CH3 domains from IgG<sub>1</sub>.

109. A non-naturally occurring single chain Fv protein comprising: i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region where leucine is replaced by serine at position 11 in the first framework region of the heavy chain  
20 variable region; wherein said protein has an increased expression or stability in mammalian cells relative to a protein not having said amino acid substitution; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-  
25 chain Fv protein is capable of at least one immunological activity.

110. A non-naturally occurring single chain Fv protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a heavy chain variable region wherein leucine  
30 at is replaced by serine at position 11 in the first framework region of the heavy chain variable region; and ii) a second polypeptide comprising an N-terminally truncated IgE

immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

111. A protein of claim 110 wherein the amino acid substitution at position 11 is effective to increase expression or stability of said single chain Fv protein relative to a single chain Fv protein without said deletion or substitution.

112. A protein of claim 110 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from IgE.

113. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma and said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE.

114. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from murine IgE.

115. A protein of claim 110 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein said heavy chain constant region comprises CH2, CH3, and CH4 domains from human IgE.

116. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

117. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv.

118. A protein of claim 116 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

119. A protein of claim 118 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

120. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 9, 10, 11, 12, 108, 110, 112.

121. A protein of claim 117 wherein said binding domain polypeptide is a single chain Fv comprising a light chain variable region, wherein said light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107.

122. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.

123. A protein of claim 122 wherein acid at position of 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

124. A protein of claim 122 wherein the amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, and glutamine.

125. A protein of claim 122 wherein leucine is replaced by serine at position 11.

126. A protein of claim 122 wherein leucine is replaced by des-leucine at position 11.

127. A protein of claim 116 wherein said binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of said single chain Fv has been deleted.

5 128. A protein of claim 121 where leucine is replaced by serine at position 106.

129. A protein of claim 117 wherein said binding domain polypeptide binds to a tumor antigen.

130. A protein of claim 117 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.

10 131. A protein of claim 117 wherein said binding domain polypeptide binds to a cancer cell antigen.

132. A protein of claim 131 wherein said cancer cell antigen is a surface antigen.

15 133. A protein of claim 131 wherein said cancer cell antigen is an intracellular antigen.

134. A protein of claim 116 wherein said binding domain polypeptide binds to a B cell antigen.

135. A protein of claim 135 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

20 136. A protein of claim 117 wherein said single chain Fv binds to a B cell antigen.

137. A protein of claim 136 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

25 138. A protein of claim 137 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

139. A protein of claim 117 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

140. A protein of claim 116 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

141. A protein of claim 136 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.

5 142. A protein of claim 116 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8, CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand),  
10 ICOS, L6, B7-H1, and HLA class II.

143. A protein of claim 116 wherein said protein is capable of forming a complex comprising two or more of said proteins.

144. A protein of claim 143 wherein said complex is a dimer.

145. A protein of claim 116 wherein said protein is a monomer.

15 146. A protein of claim 116 coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety.

147. A protein of claim 116 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active  
20 signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

148. A protein of claim 147 which is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from the group  
25 consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.

149. A protein of claim 147 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

150. A protein of claim 149 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

151. A protein of claim 147 which is capable of cellular activation,  
5 wherein said activation leads to changes in cellular transcriptional activity.

152. A protein of claim 151 wherein said cellular transcriptional activity is increased.

153. A protein of claim 151 wherein said cellular transcriptional activity is decreased.

10 154. A protein of claim 147 wherein said one or more biologically active molecules is a protease.

155. A protein of claim 147 wherein said one or more biologically active molecules is a cytokine.

156. A protein of claim 155 wherein said cytokine is selected from the  
15 group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.

157. A protein of claim 147 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.

158. A protein of claim 147 A protein which is capable of neutralization of  
20 a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.

159. A protein of claim 147 which is capable of neutralization of a toxin,  
wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin,  
cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat  
25 stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin,  
*Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.

160. A protein of claim 147 which is capable of neutralization of a toxin,  
wherein said toxin is an endotoxin selected from the group consisting of saxitoxins,  
tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins,  
30 and grayanotoxins.



161. A protein of claim 116 wherein said binding domain polypeptide comprises a light chain variable region attached to said heavy chain variable region by a binding domain linker, wherein said binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser.

5                   162. A protein of claim 116 comprising three Gly-Gly-Gly-Gly-Ser peptides.

163. A protein of claim 116 wherein said binding domain polypeptide comprises wild type or engineered immunoglobulin variable region obtained from species selected from the group consisting of human, murine, rat, pig, and monkey.

10                   164. A protein of claim 116 wherein said protein is capable of binding to an intracellular target to effect a cellular function.

15                   165. A protein of claim 116 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

166. A protein of claim 116 wherein said immunoglobulin heavy chain constant region polypeptide is without a functionally active CH1 domain.

20                   167. A protein of claim 116 said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

168. A protein of claim 116 comprising at least a portion of a human constant region.

25                   169. A protein of claim 116 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

170. A protein of claim 116 wherein said connecting region comprises a human IgE hinge or a portion thereof.

171. A protein of claim 116 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

5 172. A protein of claim 116 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

173. A protein of claim 116 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

10 174. A protein of claim 116 wherein said connecting region comprises a glycosylation site.

175. A protein of claim 116 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

176. A protein of claim 116 wherein said connecting region has one cysteine residue.

15 177. A protein of claim 116 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

178. A protein of claim 116 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

20 179. A protein of claim 116 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

25 180. A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 domain.

181. A protein of claim 179 where said single chain protein comprises a CTLA-4 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 domains from IgG<sub>1</sub>.

5 182. A protein of claim 179 where said single chain protein comprises a FC2-2 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

10 183. A protein of claim 179 where said single chain protein comprises a UCHL-1 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

15 184. A protein of claim 179 where said single chain protein comprises a 5B9 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

20 185. A protein of claim 179 where said single chain protein comprises a 2H7 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

25 186. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

30 187. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein phenylalanine is replaced by tyrosine at position 405 in said CH3 region.

188. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprises CH2 and CH3 domains from IgG<sub>1</sub> wherein phenylalanine is replaced by alanine at position 405 in said CH3 region.

189. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein tyrosine is replaced by alanine at position 407 in said CH3 region.

190. A protein of claim 189 having an apparent molecular weight of about 75 kDa.

191. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407 in said CH3 region.

192. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

193. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

194. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first and second cysteine residues are replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

195. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said second cysteine residue is replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

5 196. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv comprising a connecting region wherein said third cysteine residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

10 197. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain, wherein in the connecting region said first cysteine residue is replaced by serine, and where said heavy chain constant region comprises 2H7 single chain Fv CH2 and CH3 domains.

15 198. A protein of claim 179 where said single chain protein comprises a HD37 single chain Fv binding domain, wherein in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

199. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG<sub>1</sub>.

20 200. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG<sub>2</sub>.

25 201. A protein of claim 179 where said single chain protein comprises a 2H7 single chain Fv binding domain and said heavy chain constant region comprises CH2 and CH3 constant region polypeptides from llama IgG<sub>3</sub>.

30 202. A protein of claim 179 where said single chain protein comprises a CD-16-6 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region.

203. A protein of claim 179 where said single chain protein comprises a CD-16 receptor binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein  
5 proline is replaced by serine at position 238 in said CH2 region.

204. A protein of claim 179 where said single chain protein comprises a 2e12 single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>  
10 wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

205. A protein of claim 179 where said single chain protein comprises a 10A8 scFv single chain Fv binding domain, wherein in the connecting region said first, second, and third cysteine residues are replaced by serine and said proline residue is  
15 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

20 206. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 40.2.36 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region, and  
25 wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

207. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is  
30 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

208. A protein of claim 179 where said single chain protein comprises a  
5 single chain Fv binding domain from a G19-4 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region, and wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and  
10 cytoplasmic tail domains.

209. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a G19-4 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

210. A protein of claim 179 where said single chain protein comprises a  
15 single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising CD80  
20 transmembrane and cytoplasmic tail domains.

211. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains  
25 from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

212. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said  
30 first, second and third cysteine residues are replaced by serine and said proline residue is

replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

213. A protein of claim 179 where said single chain protein comprises a  
5 single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

10 214. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said  
15 CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

215. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is  
20 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

216. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said  
25 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.



217. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

218. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

219. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

220. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

221. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

222. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains  
5 from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

223. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 scFv hybridoma, where in the connecting region  
10 said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

224. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said  
15 first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and  
20 cytoplasmic tail domains.

225. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains  
25 from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising mFADD transmembrane and cytoplasmic tail domains.

226. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is  
30 replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains

from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

227. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp3 transmembrane and cytoplasmic tail domains.

228. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

229. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising mcasp8 transmembrane and cytoplasmic tail domains.

230. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

231. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said

first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp3 transmembrane and cytoplasmic tail domains.

232. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>, wherein said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

233. A protein of claim 179 where said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub> wherein proline is replaced by serine at position 238 in said CH2 region and said CH3 domain is attached to a polypeptide comprising hcasp8 transmembrane and cytoplasmic tail domains.

234. A protein of claim 179 where said single chain protein comprises a L6 single chain Fv binding domain, where in the connecting region said first, second and third cysteine residues are replaced by serine and said proline residue is replaced by serine, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

235. A protein of claim 179 where said single chain protein comprises a G28-1 single chain Fv comprising a connecting region wherein said first, second, and third cysteine residues are replaced by serine and said proline residue is replaced by serine, where said heavy chain constant region comprises G28-1 single chain Fv CH2 and CH3 domains.

236. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD comprising the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell

population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

237. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a protein that is less than about 150kD consisting essentially of the steps of a) treating the target cell population with a first protein or peptide that binds to cells within said target cell population, and b) treating the target cell population with a second protein or peptide that capable of at least one of i) binding an Fc receptor, ii) inducing target cell apoptosis, or iii) fix complements, wherein said first protein or peptide molecule is directly connected to said second protein or peptide molecule or, optionally, said first protein or peptide molecule and said second protein or peptide molecule are linked by a third protein or peptide molecule, and wherein said protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope.

238. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution; ii) a second polypeptide comprising a connecting region attached to said first polypeptide; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

239. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, said binding domain polypeptide comprising a light chain variable region comprising one or more amino acid deletion or substitution in positions 12, 80, 81, 83, 105, 106, and 107; ii) a

second polypeptide comprising a connecting region attached to said first polypeptide; and  
iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain  
constant region polypeptide attached to the second polypeptide, wherein said non-naturally  
occurring single-chain protein is capable of at least one immunological activity.

5                   240. A protein of claim 238 wherein the one or more amino acid  
substitution or deletion in said light chain variable region is effective to increase expression  
or stability of said protein relative to a protein without said deletion or substitution.

                  241. A protein of claim 238 wherein said binding domain polypeptide is a  
single chain Fv.

10                  242. A protein of claim 238 wherein said binding domain polypeptide  
comprises an immunoglobulin light chain variable region polypeptide and an  
immunoglobulin heavy chain variable region polypeptide.

                  243. A protein of claim 242 further comprising a second binding domain  
polypeptide capable of binding a second target molecule, said second binding domain  
15 polypeptide comprising an immunoglobulin light chain variable region polypeptide and an  
immunoglobulin heavy chain variable region polypeptide.

                  244. A protein of claim 241 wherein said binding domain polypeptide is a  
single chain Fv and said light chain variable region of said single chain Fv has an amino acid  
deletion or substitution at position 106.

20                  245. A protein of claim 241 wherein said binding domain polypeptide is a  
single chain Fv and the amino acid at position 106 of the heavy chain variable region of said  
single chain Fv has been deleted.

                  246. A protein of claim 238 wherein said binding domain polypeptide is a  
single chain Fv and said light chain variable region of said single chain Fv has an amino acid  
25 substitution at position 106.

                  247. A protein of claim 244 wherein leucine is replaced by serine at  
position of 106.

                  248. A protein of claim 244 wherein leucine is replaced by des-leucine at  
position of 106.

249. A protein of claim 244 wherein the amino acid substituted for the amino acid at position 106 of the single chain Fv light chain variable region is selected from the group consisting of serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

5                    250. A protein of claim 244 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.

251. A protein of claim 241 wherein said binding domain polypeptide binds to a tumor antigen.

10                   252. A protein of claim 241 wherein said binding domain polypeptide binds to an antigen on an immune effector cell.

253. A protein of claim 241 wherein said binding domain polypeptide binds to a cancer cell antigen.

15                   254. A protein of claim 253 wherein said cancer cell antigen is a surface antigen.

255. A protein of claim 253 wherein said cancer cell antigen is an intracellular antigen.

256. A protein of claim 238 wherein said binding domain polypeptide binds to a B cell antigen.

20                   257. A protein of claim 256 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

258. A protein of claim 241 wherein said single chain Fv binds to a B cell antigen.

25                   259. A protein of claim 258 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, CD80, and CD86.

260. A protein of claim 259 wherein said single chain Fv is selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

30                   261. A protein of claim 238 wherein said binding domain polypeptide is an scFv that binds to a B cell differentiation antigen.

262. A protein of claim 261 wherein said B cell antigen is selected from the group consisting of CD19, CD20, CD22, CD37, and CD40.

263. A protein of claim 238 wherein said binding domain polypeptide binds to a target selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD8,  
5 CD10, CD11b, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II.

264. A protein of claim 238 wherein said protein is capable of forming a  
10 complex comprising two or more of said proteins.

265. A protein of claim 264 wherein said complex is a dimer.

266. A protein of claim 238 wherein said protein is a monomer.

267. A protein of claim 238 wherein said immunological activity is selected from the group consisting of antibody dependent cell-mediated cytotoxicity,  
15 complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin.

268. A protein of claim 267 which is capable of induction of biologically  
20 active signals by activation or inhibition of one or more molecules selected from the group consisting of protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components.

269. A protein of claim 267 which is capable of induction of one or more immune effector cells selected from the group consisting of NK cells, monocytes,  
25 macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils.

270. A protein of claim 267 wherein said induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules.

271. A protein of claim 267 which is capable of cellular activation,  
30 wherein said activation leads to changes in cellular transcriptional activity.



272. A protein of claim 271 wherein said cellular transcriptional activity is increased.
273. A protein of claim 271 wherein said cellular transcriptional activity is decreased.
- 5 274. A protein of claim 267 wherein said one or more biologically active molecules is a protease.
275. A protein of claim 267 wherein said one or more biologically active molecules is a cytokine.
- 10 276. A protein of claim 275 wherein said cytokine is selected from the group consisting of monokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins.
277. A protein of claim 267 which is capable of neutralization of an infectious agent, wherein said infectious agent is a bacterium, a virus, a parasite, or a fungus.
- 15 278. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is selected from the group consisting of endotoxins and exotoxins.
279. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an exotoxin selected from the group consisting of anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin LT, *E. coli* heat stable toxin ST, shiga toxin *Pseudomonas* Exotoxin A, botulinum toxin, tetanus toxin, 20 *Bordetella pertussis* AC toxin, and *Bacillus anthracis* EF.
280. A protein of claim 267 which is capable of neutralization of a toxin, wherein said toxin is an endotoxin selected from the group consisting of saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins.
- 25 281. A protein of claim 238 wherein said protein is capable of binding to an intracellular target to effect a cellular function.
282. A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region 30 polypeptide.

283. A protein of claim 238 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

5                   284. A protein of claim 238 comprising at least a portion of a human constant region.

285. A protein of claim 238 wherein said connecting region comprises a naturally occurring hinge region selected from the group consisting of a human hinge or portion thereof, human IgG hinge or a portion thereof, human IgA hinge or a portion thereof, human IgE hinge or a portion thereof, camelid hinge region or a portion thereof, IgG1 llama hinge region or portion thereof, nurse shark hinge region or portion thereof, and spotted ratfish hinge region or a portion thereof.

286. A protein of claim 238 wherein said connecting region comprises a human IgE hinge or a portion thereof.

15                   287. A protein of claim 238 wherein said connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue.

288. A protein of claim 238 wherein said connecting region comprises a human IgGA hinge region having between zero and two cysteine residues.

20                   289. A protein of claim 238 wherein said connecting region comprises a wild type human IgG1 immunoglobulin hinge region.

290. A protein of claim 238 wherein said connecting region comprises a glycosylation site.

291. A protein of claim 238 wherein said connecting region has no cysteine residues capable of forming disulfide bonds.

25                   292. A protein of claim 238 wherein said connecting region has one cysteine residue.

293. A protein of claim 238 wherein said connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising no more than one cysteine residue.

294. A protein of claim 238 wherein said connecting region is altered so that said protein has a reduced ability to dimerize.

295. A protein of claim 238 wherein said connecting region comprises a proline and first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, said third cysteine residue is N-terminal to said proline residue.

296. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprises at least a portion of an IgA hinge region, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

297. A protein of claim 296 wherein said binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

298. A protein of claim 297 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

299. A protein of claim 296 wherein said connecting region consists essentially of at least a portion of an IgA hinge region.

300. A protein of claim 296 wherein said connecting region consists essentially of an IgA hinge region.

301. A protein of claim 300 that is expressed as a protein having an apparent molecular weight of at least about 70 kDa.

302. A protein of claim 300 that is expressed as a protein having an apparent molecular weight of between about 70 kDa and about 700 kDa.

303. A protein of claim 296 wherein said connecting region comprises between 10 to 50 amino acids.

304. A protein of claim 296 wherein said connecting region comprises a modified human IgA immunoglobulin hinge region polypeptide.

305. A protein of claim 296 wherein said connecting region has only one cysteine residue.

5 306. A protein of claim 296 wherein said connecting region two cysteine residues.

307. A protein of claim 296 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide in the third polypeptide comprises IgA immunoglobulin heavy chain constant regions.

10 308. A protein of claim 307 comprising CH2 and CH3 domains from IgA immunoglobulin heavy chain constant regions.

309. A protein of claim 308 wherein said IgA CH3 constant region domain comprises a substitution or deletion in one or more amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide.

15 310. A protein of claim 308 wherein said IgA heavy chain constant region is capable of associating with a J chain polypeptide.

311. A protein of claim 309 wherein said IgA CH3 region domain comprises a deletion in between four and twenty amino acids.

20 312. A protein of claim 309 wherein said IgA region domain comprises a deletion of four amino acids.

313. A protein of claim 309 wherein said IgA region domain comprises a deletion of twenty amino acids.

25 314. A protein of claim 296 wherein said single chain protein comprises a L6 single chain Fv binding domain, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

30 315. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a L6 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

316. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

5 317. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a CTLA-4 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA and said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of  
10 associating with a J chain polypeptide.

318. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from murine IgA.

15 319. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in eighteen amino acids that render the IgA heavy chain constant region incapable of associating with a J chain  
20 polypeptide.

320. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a IgA hinge or portion thereof, and said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino  
25 acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

321. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12 hybridoma, said connecting region comprises a  
30 IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3

domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

5                    322. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 1D8 hybridoma, said connecting region comprises a IgA hinge or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of associating with a J chain  
10 polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

                  323. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a G28-1 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region  
15 comprising CH2 and CH3 domains from IgG<sub>1</sub>.

                  324. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprising CH2 and CH3 domains from IgG<sub>1</sub>.

20                    325. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA, wherein said CH3 comprises a deletion or substitution in four amino acids that render the IgA heavy chain constant region incapable of  
25 associating with a J chain polypeptide and said CH3 domain is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

                  326. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a 2H7 hybridoma, wherein the connecting region comprises an IgA hinge region or portion thereof, said heavy chain constant region

comprises CH2 and CH3 domains from IgA, wherein the IgA heavy chain constant region is capable of associating with a J chain polypeptide.

327. A protein of claim 296 wherein said single chain protein comprises a single chain Fv binding domain from a HD37 hybridoma, wherein the connecting region  
5 comprises an IgA hinge region or portion thereof, said heavy chain constant region comprises CH2 and CH3 domains from IgA.

328. A non-naturally occurring single chain protein comprising i) a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, and ii) a second polypeptide comprising at least a portion of an IgE  
10 immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

329. A protein of claim 328 wherein said binding domain polypeptide comprises a non-IgE variable region.

15 330. A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, 1D8, and 4.4.220 single chain Fv.

331. A protein of claim 328 wherein said binding domain polypeptide  
20 comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

332. A protein of claim 331 further comprising a second binding domain polypeptide capable of binding a second target molecule, said second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an  
25 immunoglobulin heavy chain variable region polypeptide.

333. A protein of claim 332 wherein said immunoglobulin light and heavy chains comprise IgG immunoglobulin light and heavy chains or a portions thereof.

334. A protein of claim 328 wherein said binding domain polypeptide comprises at least a portion of an IgG immunoglobulin variable region.

335. A protein of claim 334 wherein said IgG variable region comprises a heavy chain variable region and a light chain variable region.

336. A protein of claim 328 that is capable of binding to a Fc receptor specific for IgE.

5 337. A protein of claim 328 that does not have IgE variable chain region.

338. A protein of claim 328 wherein the binding domain consist essentially of an IgG immunoglobulin light chain region and a IgG immunoglobulin light and heavy chain region.

10 339. A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2 and CH3 domains.

340. A protein of claim 328 wherein said IgE immunoglobulin heavy chain constant region polypeptide comprises CH2, CH3 and CH4 domains.

15 341. A protein of claim 328 wherein said binding domain polypeptide is a single chain Fv comprising a heavy chain variable region, wherein said heavy chain variable region has an amino acid substitution at amino acid position 11.

342. A protein of claim 328 wherein said single chain protein is a single chain Fv selected from the group consisting of HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv.

20 343. A protein of claim 328 wherein said single chain protein is a single chain Fv comprising a 2H7 single chain Fv or a G28-1 single chain Fv.

25 344. A method of reducing a target cell population in a subject comprising administering to said subject a therapeutically effective amount of a non-naturally occurring single chain protein, said protein comprising a first polypeptide comprising a binding domain polypeptide, said binding domain polypeptide comprising at least a portion of an IgG variable region and being capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.



345. The method of claim 344 wherein said protein is administered into the blood stream of a patient.

346. The method of claim 344 wherein monocytes are activated but mast cells are not activated as a result of administration of the protein.

5 347. The method of claim 344 wherein the protein is administered or coadministered with a histamine release blocker.

348. A method of depleting cells in an animal comprising administering a modified IgE protein into the blood stream of an animal.

10 349. The method of claim 348 wherein the modified IgE protein is administered or coadministered with a histamine release blocker.

350. A protein of claim 328 wherein said connecting region comprises a IgG hinge or portion thereof, and said heavy chain constant region is from IgE and comprises CH3 and CH4 domains without a CH2 domain.

15 351. A protein of claim 328 wherein said single chain protein comprises a single chain Fv binding domain from a 2e12, and said heavy chain constant region comprises IgE CH2, CH3, and CH4 domains, and wherein said heavy chain constant region is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

20 352. A non-naturally occurring single chain protein comprising i) a first polypeptide having a binding domain polypeptide capable of binding to a target molecule; ii) a second polypeptide comprising a connecting region attached to said first polypeptide, said connecting region comprising three cysteine residues and one proline residue, wherein one or more of said cysteine residues is deleted or substituted; and iii) a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said non-naturally occurring single-  
25 chain protein is capable of at least one immunological activity.

30 353. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not substituted or deleted, and where said second and third cysteine are substituted or deleted.

354. A protein of claim 353 wherein said second and third cysteine residues are substituted by another amino acid.

355. A protein of claim 354 wherein said second and third cysteine residues are substituted by serine residues.

5 356. A protein of claim 352 that is expressed as a homogeneous protein having an apparent molecular of about 75 kDa.

357. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein  
10 said first cysteine residue is substituted or deleted.

358. A protein of claim 352 where said second and third cysteine residues in said connecting region are not substituted or deleted.

359. A protein of claim 352 wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein  
15 said second cysteine residue is substituted or deleted.

360. A protein of claim 352 wherein said first and third cysteine residues in said connecting region are not substituted or deleted.

361. A protein of claim 352 wherein said connecting region comprises  
20 first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said third cysteine residue is substituted or deleted.

362. The protein of claim 10 where said first and second cysteine residues in said connecting region are not substituted or deleted.

25 363. A protein of claim 352 wherein said connecting region comprises between 5 and 65 amino acids.

364. A protein of claim 352 wherein said connecting region comprises between 10 and 50 amino acids.

365. A protein of claim 352 wherein said connecting region comprises  
30 between 15 and 35 amino acids.

366. A protein of claim 352 wherein said connecting region comprises at least five consecutive hinge region amino acids.

367. A protein of claim 352 wherein said connecting region comprises at least ten consecutive hinge region amino acids.

5 368. A protein of claim 352 wherein said connecting region comprises at least fifteen consecutive hinge region amino acids.

369. A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG<sub>1</sub> hinge region.

10 370. A protein of claim 369 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG<sub>1</sub> hinge region.

371. A protein of claim 353 wherein said second and third cysteines are replaced by serine residues and said connecting region comprises at least a portion of a IgG<sub>2</sub> hinge region.

15 372. A protein of claim 371 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG<sub>2</sub> hinge region.

373. A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG<sub>3</sub> hinge region.

20 374. A protein of claim 373 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG<sub>3</sub> hinge region.

375. A protein of claim 353 wherein said second and third cysteine residues are replaced by serine residues and said connecting region comprises at least a portion of a IgG<sub>4</sub> hinge region.

25 376. A protein of claim 375 wherein said connecting region comprises at least 5 consecutive amino acids of an IgG<sub>4</sub> hinge region.

377. A protein of claim 352 wherein said second and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is

replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

378. A protein of claim 352 wherein said first and third cysteine residues are substituted or deleted and said proline residue is substituted or deleted, wherein said  
5 heavy chain constant region polypeptide comprises a CH3 domain wherein tyrosine is replaced by alanine at position 407, and wherein said protein has an apparent molecular weight of about 75kDa.

379. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target  
10 molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, wherein said connecting region comprises first, second, and third cysteine residues, where said first cysteine residue is N-terminal to said second cysteine and said second cysteine is N-terminal  
15 to said third cysteine, wherein one or both of said second and third cysteine residues is substituted or deleted, and wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

380. A protein of claim 352 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide.

20 381. A protein of claim 352 wherein said connecting region has only one cysteine residue.

382. A protein of claim 381 wherein said connecting region comprises a modified human IgG1 immunoglobulin hinge region polypeptide.

383. A protein of claim 352 wherein said N-terminally truncated  
25 immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

384. A protein of claim 352 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2

constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.

385. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion that inhibits two of said proteins from associating together to form a non covalent dimer, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

386. A non-naturally occurring single chain protein, including a first polypeptide comprising a binding domain polypeptide capable of binding to a target molecule, a second polypeptide comprising a connecting region attached to said first polypeptide, a third polypeptide comprising an immunoglobulin heavy chain CH2 constant region polypeptide and an immunoglobulin heavy chain CH3 constant region polypeptide, said CH3 constant region polypeptide comprising one or more amino acid substitution or deletion at positions 405 and 407, wherein said non-naturally occurring single-chain protein is capable of at least one immunological activity.

387. A protein of claim 252 or 253 comprising amino acid substitutions at position 405 and position 407.

388. A protein of claim 387 wherein phenylalanine is replaced with alanine at position 405 and tyrosine is substituted with alanine at position 407.

389. A protein of claim 387 having an apparent molecular weight of between about 40 kDa and about 50 kDa.

390. A protein of claim 387 that has a biological activity of an agonist or antagonist.

391. A protein of claim 390 that has a biological activity of an antagonist.

392. A protein of claim 387 wherein only one amino acid at positions 405 and 407 is substituted.

393. A protein of claim 392 having an apparent molecular weight of between about 70 kDa and about 80 kDa.
394. A protein of claim 387 where phenylalanine is replaced by alanine at position 405.
- 5 395. A protein of claim 387 where phenylalanine is replaced by tyrosine at position 405.
396. A protein of claim 386 where tyrosine is replaced by alanine at position 407.
- 10 397. A protein of claim 386 comprising amino acid substitutions at position 405 and position 407.
398. A protein of claim 393 wherein phenylalanine is replaced by alanine at position 405 and tyrosine is replaced by alanine at position 407.
399. A polynucleotide that encodes a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109.
- 15 400. A polynucleotide that encodes a protein according to any one of claims 116, 120, 121, and 180-235.
401. A polynucleotide that encodes a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386.
- 20 402. A polynucleotide of any one of claims 399-401 that is operably linked to a promoter or other sequence that enhances expression of the polynucleotide in a cell.
403. A cell containing a polynucleotide of any one of claims 399-401.
404. A recombinant vector capable of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109.
- 25 405. A recombinant vector capable of expressing a protein according to any one of claims 116, 120, 121, and 180-235.
406. A method of expressing a protein according to any one of claims 1, 8, 9, 12, 18, 30, and 112-109 under conditions in which the protein is expressed.
407. A method of expressing a protein according to any one of claims 116, 120, 121, and 180-235 under conditions in which the protein is expressed.

408. A method of expressing a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 under conditions in which the protein is expressed.

409. A composition comprising a protein according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386 in combination with one or more additional  
5 therapeutic compounds.

410. A method of treating a subject comprising the steps of administering to said subject a one or more first compound according to any one of claims 238, 239, 296, 328, 352, 353, 385, and 386, administering or coadministering a second compound to the subject in combination said first compound, wherein the treatment is effective to treat or  
10 prevent a disease, ailment, or undesired condition.

411. The method according to claim 410 wherein the second compound is selected from the group consisting of a chemotherapeutic compound, therapeutic drug, angiogenesis inhibitors, steroids, B-cell activators, T-cell activators, colony stimulating factors, tumor necrosis factors, interferons, antibody, binding construct of the invention,  
15 gene therapy, retinoids, surgical procedures, alkylating agents, nucleoside analogues, topoisomerase II, VEGF, IFN-alpha, DMAR agents, interleukin-1, glucocorticoids, p38 inhibitors, interleukin-4, interleukin-6, interleukin-2, interleukin-12, IFN- $\gamma$ , GM-CSF, G-CSF, M-CSF, anti-CTLA-4, Bcl-2 antisense, vitamin A, anti-CD19, anti-CD20, anti-CD22, anti-CD28 or anti-CD3.

20

412. A method for displaying recombinant molecules, which molecules include a native or engineered immunoglobulin heavy chain variable region, the improvement comprising a immunoglobulin heavy chain region that includes one or more mutation, substitution, alteration, and/or deletion at one or more amino acid residue  
25 corresponding positions 9, 10, 11, 12, 108, 110, and 112 in said heavy chain variable region.

413. A non-naturally occurring single chain antigen-binding protein comprising protein having a formula selected from the group consisting of 2H7 scFv VH L11S (CSC-S) H WCH2 WCH3, 2H7 scFv VH L11S IgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WlgACH2 T4CH3, 2H7 scFv VH  
30 L11S (SSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3,

2H7 scFv VH L11S (SSS-S) H P331S CH2 WCH3, 2HU scFv VH L11S (CSS-S) H P331S  
 CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH L11S  
 (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q  
 CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H  
 5 WCH2 WCH3, G28-1 scFv IgAH WCH2 WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2  
 WCH3, G28-1 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (CSC-S) H  
 WCH2 WCH3, G28-1 scFv VH L11S (SSC-P) H WCH2 WCH3, CTLA4 (SSS-S) H  
 P238SCH2 WCH32, CTLA4 (CCC-P) WH WCH2 WCH3, FC2-2 scFv (SSS-S) H WCH2  
 WCH3, FC2-2 scFv VHL11S (SSS-S) H WCH2 WCH3, UCHL-1 scFv (SSS-S) H WCH2  
 10 WCH3, UCHL-1 scFv VHL11S (SSS-S) H WCH2 WCH3, 5B9 scFv (SSS-S) H WCH2  
 WCH3, 5B9 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 WCH3,  
 2H7 scFv (SSS-S) H P238SCH2 WCH3, 2H7 scFv IgAH WCH2 WCH3, 2H7 scFv IgAH  
 WIgACH2 T4CH3, 2H7 scFv IgAH WIgACH2 WCH3 + JChain, 2H7 scFv (CCC-P) WH  
 WCH2 WCH3, 2H7 scFv (SSS-S) H WCH2 F405YCH3, 2H7 scFv (SSS-S) H WCH2  
 15 F405ACH3, 2H7 scFv (SSS-S) H WCH2 Y407ACH3, 2H4 scFv (SSS-S) HWCH2 F405A,  
 Y407ACH3, 2H7 scFv (CSS-S) H WCH2 WCH3, 2H7 scFv (SCS-S) H WCH2 WCH3,  
 2H7 scFv (SSC-P) H WCH2 WCH3, 2H7 scFv (CSC-S) H WCH2 WCH3, 2H7 scFv (CCS-  
 P) H WCH2 WCH3, 2H7 scFv (SCC-P) H WCH2 WCH3, 2H7 scFv VH L11S (SSS-S) H  
 WCH2 WCH3, 2H7 scFv VH L11S (CSS-S) H WCH2 WCH3, G28-1 scFv VH L11S (SCS-  
 20 S) H WCH2 WCH3, G28-1 scFv VH L11S (CCS-P) H WCH2 WCH3, G28-1 scFv VH  
 L11S (SCC-P) H WCH2 WCH3, G28-1 scFv VH L11S mIgE CH2 CH3 CH4, G28-1 scFv  
 VH L11S mIgAH WIgACH2 T4CH3, G28-1 scFv VH L11S hIgE CH2 CH3 CH4, G28-1  
 scFv VH L11S hIgAH WIgACH2 T4CH3, HD37 scFv IgAH WCH2 WCH3, HD37 scFv  
 (SSS-S) H WCH2 WCH3, HD37 scFv VH L11S (SSS-S) H WCH2 WCH3, L6 scFv IgAH  
 25 WCH2 WCH3, L6 scFv VHL11S (SSS-S) H WCH2 WCH3, 2H7 scFv-llama IgG1, 2H7  
 scFv-llama IgG2, 2H7 scFv-llama IgG3, CD16-6 low (ED)(SSS-S) H P238SCH2 WCH3,  
 CD16-9 high (ED)(SSS-S) H P238SCH2 WCH3, 2e12 scFv (SSS-s)H P238SCH2 WCH3—  
 hCD80TM/CT, 10A8 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 40.2.36 scFv  
 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2H7 scFv (SSS-s)H P238SCH2 WCH3—  
 30 hCD80TM/CT, G19-4 scFv (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 2e12 scFv (SSS-



s)H WCH2 WCH3—hCD80TM/CT, 2e12 scFv IgAH IgACH2 T4CH3—hCD80TM/CT,  
 2e12 scFv IgE CH2CH3CH4—hCD80TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—  
 mFADD-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 2e12 scFv (SSS-  
 s)H WCH2 WCH3—mcasep3-TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—mcasep3-  
 5 TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—mcasep8--TM/CT, 2e12 scFv (SSS-s)H  
 P238SCH2 WCH3—mcasep8-TM/CT, 2e12 scFv (SSS-s)H WCH2 WCH3—hcasep3--  
 TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasep3-TM/CT, 2e12 scFv (SSS-s)H  
 WCH2 WCH3—hcasep8--TM/CT, 2e12 scFv (SSS-s)H P238SCH2 WCH3—hcasep8-  
 TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—hCD80TM/CT, 1D8 scFv--hIgG1  
 10 (SSS-s)H WCH2 WCH3—hCD80TM/CT, 1D8 scFv—mIgAT4—hCD80TM/CT, 1D8  
 scFv--hIgE—hCD80TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mFADD-  
 TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mFADD-TM/CT, 1D8 scFv--hIgG1  
 (SSS-s)H WCH2 WCH3—mcasep3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2  
 WCH3—mcasep3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—mcasep8--TM/CT,  
 15 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—mcasep8-TM/CT, 1D8 scFv--hIgG1 (SSS-  
 s)H WCH2 WCH3—hcasep3--TM/CT, 1D8 scFv--hIgG1 (SSS-s)H P238SCH2 WCH3—  
 hcasep3-TM/CT, 1D8 scFv--hIgG1 (SSS-s)H WCH2 WCH3—hcasep8--TM/CT, 1D8 scFv--  
 hIgG1 (SSS-s)H P238SCH2 WCH3—hcasep8-TM/CT, L6 scFv (SSS-S) H WCH2 WCH3,  
 2H7 scFv CD154 (L2), 2H7 scFv CD154 (S4), CTLA4 IgAH IGACH2CH3, CTLA4 IgAH  
 20 IgACH2 T4CH3, 2H7 scFv IgAH IgACH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2H&-  
 40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-cd40), 2H7 scFv IgAH  
 IgACH2 T4CH3-hCD89 TM/CT, G19-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT,  
 and 2e12 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT.

25

30

## ABSTRACT

The invention relates to novel binding domain-immunoglobulin fusion proteins that feature a binding domain for a cognate structure such as an antigen, a counterreceptor or the like, a wild-type IgG1, IGA or IgE hinge-acting region, *i.e.*, IgE CH2, region polypeptide or a mutant IgG1 hinge region polypeptide having either zero, one or two cysteine residues, and immunoglobulin CH2 and CH3 domains, and that are capable of ADCC and/or CDC while occurring predominantly as polypeptides that are compromised in their ability to form disulfide-linked multimers. The fusion proteins can be recombinantly produced at high expression levels. Also provided are related compositions and methods, including cell surface forms of the fusion proteins and immunotherapeutic applications of the fusion proteins and of polynucleotides encoding such fusion proteins.

C:\301916\_2.DOC

Fig. 1

2H7scFv-Ig cDNA and predicted amino acid sequence:

```

HindIII      NcoI                2H7 VL Leader Peptide→
~~~~~      ~~~~~
      M D F Q V Q I F S F L L I S A S
1  AAGCTTGCCG CCATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                     2H7 VL→
      V I I A R G Q I V L S Q S P A I L S A S
61  GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121  CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                     BamHI
                                     ~~~~~
      Y Q Q K P G S S P K P W I Y A P S N L A
181  TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

      S G V P A R F S G S G S G T S Y S L T I
241  TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301  AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker
      P T F G A G T K L E L K G G G G S G G G
361  CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                     2H7 VH→
      G S G G G G S S Q A Y L Q Q S G A E L V
421  GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481  AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541  AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

      P G N G D T S Y N Q K F K G K A T L T V
601  CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661  GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721  GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

```

Fig. 1 (continued)

BclI  
~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAACCTA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

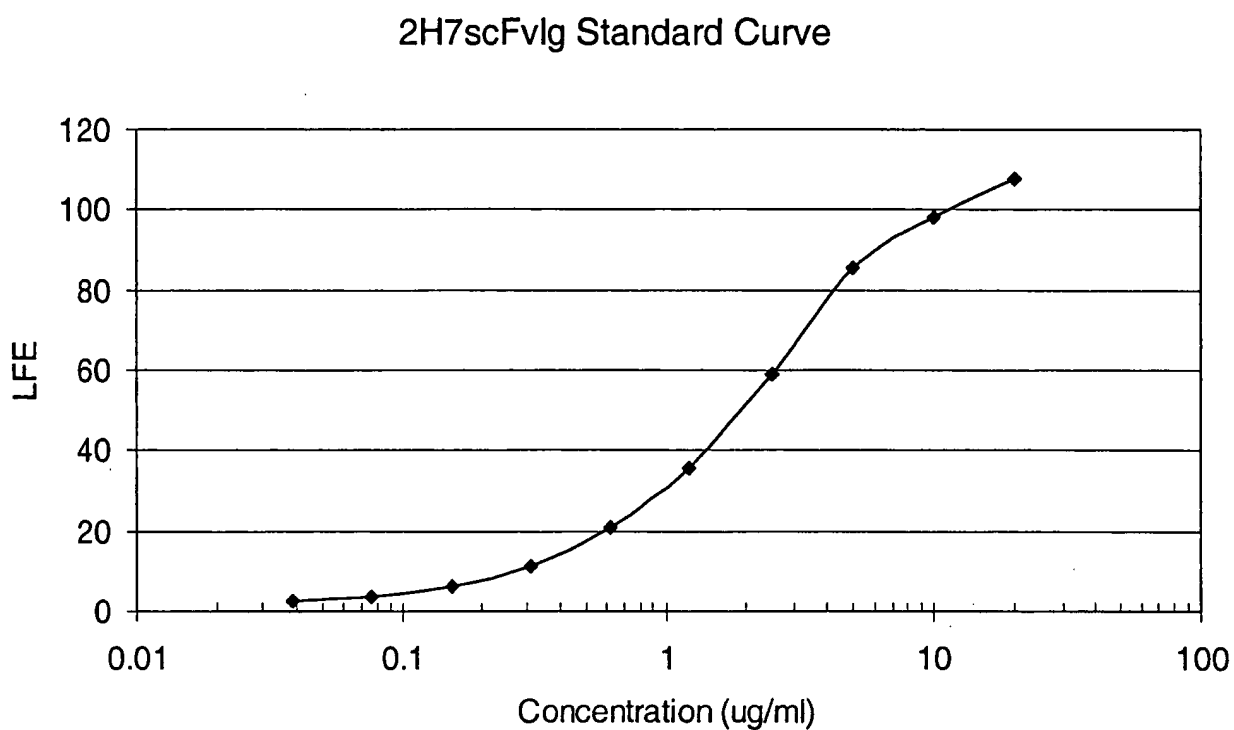
1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI  
~~~~~

1501 P G K \* S R  
CCGGGTAAAT GATCTAGA

Fig. 2  
Production Levels of 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3  
by Stable CHO Lines



Clone	LFE @ 1:50 Estimated Concentration ( $\mu\text{g/ml}$ )
D2	26.156
IIIC6	25.755
IVA3	28.661
Spent bulk	29.664

Fig. 3  
SDS-PAGE Analysis of  
2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 Protein.

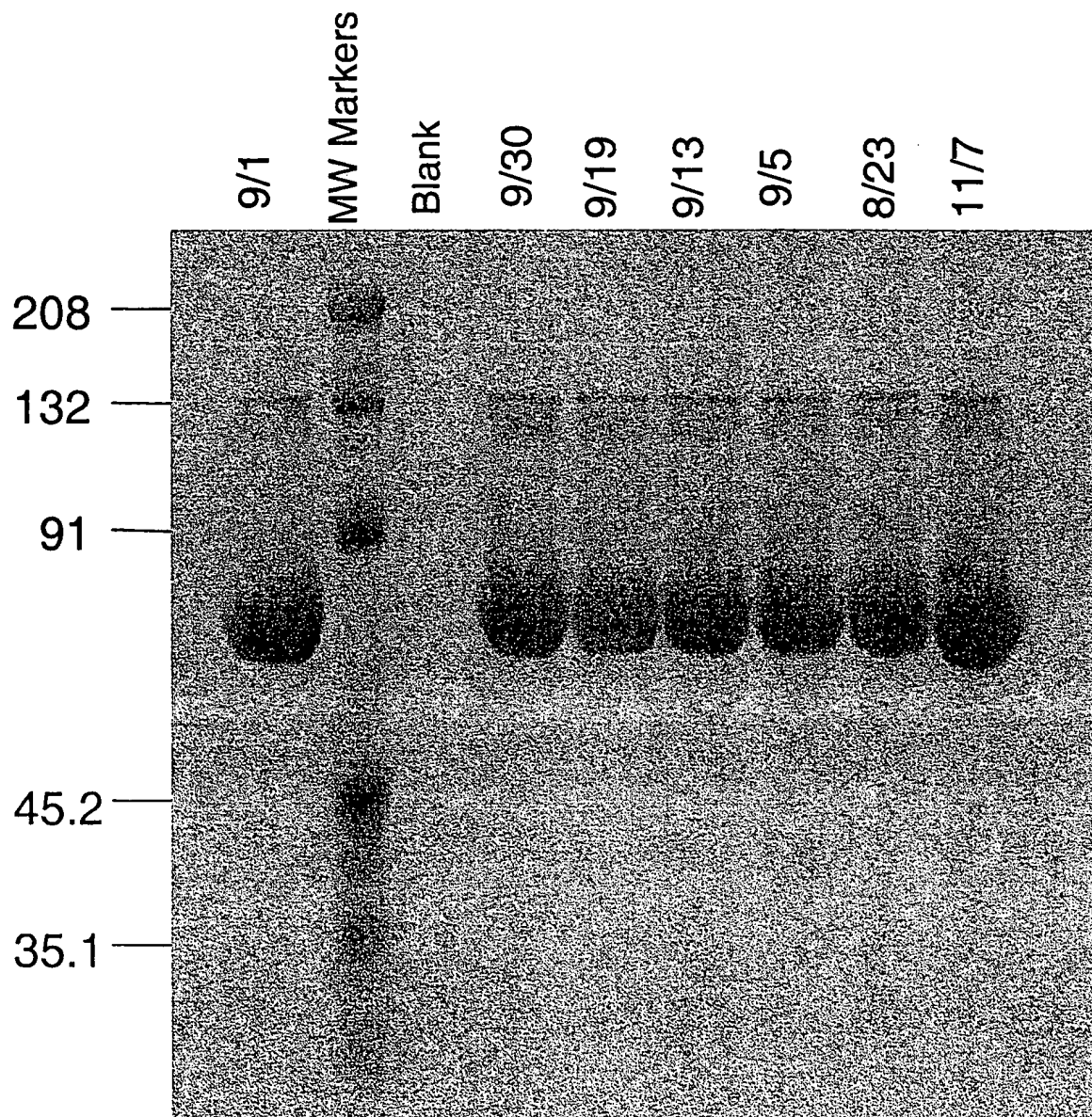


Fig. 4A

Complement Mediated B Cell Killing After Binding of  
CD20-targeted 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3:

2H7scFv-Ig Concentration	RAMOS # live cells/total cells	BJAB # live cells/total cells
20 µg/ml + complement	- 0.16	- 0.07
5 µg/ml + complement	- 0.2	- N.D.
1.25 µg/ml + complement	- 0.32	- 0.1
Complement alone	- 0.98	- 0.94

\*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

\*\*N.D. (not determined).

Fig. 4B

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-IgG1  
(SSS-S)H WCH2 WCH3:

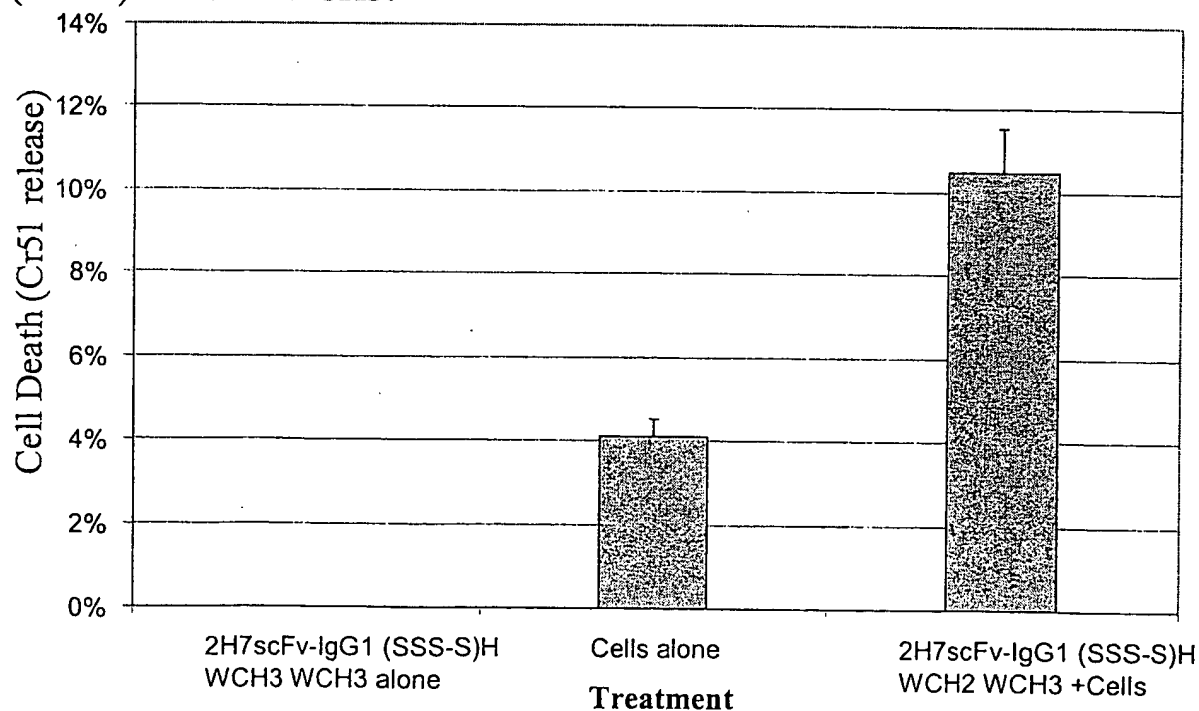


Fig. 5

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors  
on B Cell Proliferation:

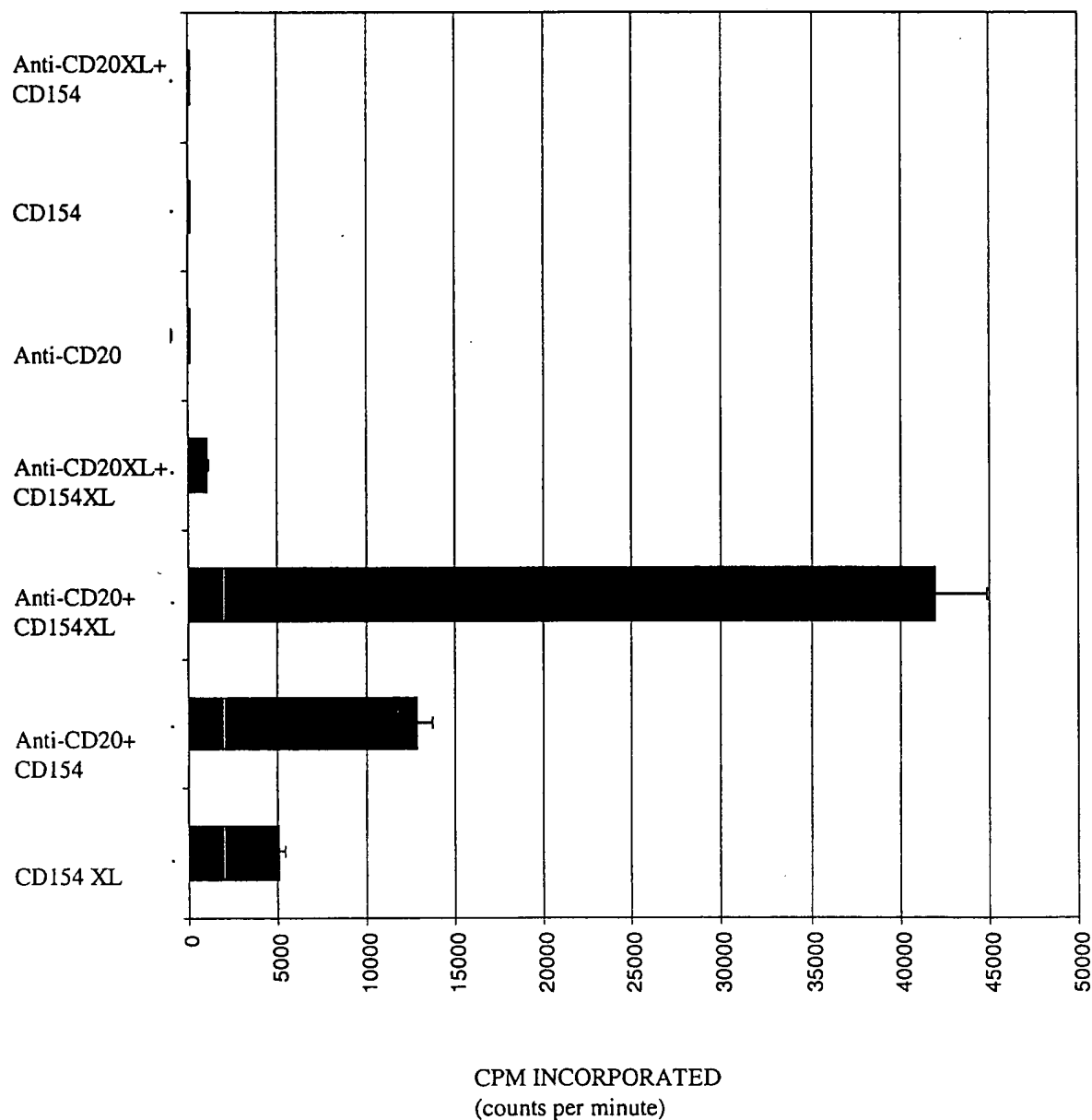




Fig. 6

Effect of Simultaneous ligation of CD20 and CD40  
on CD95 and apoptosis.

Fig. 6A.

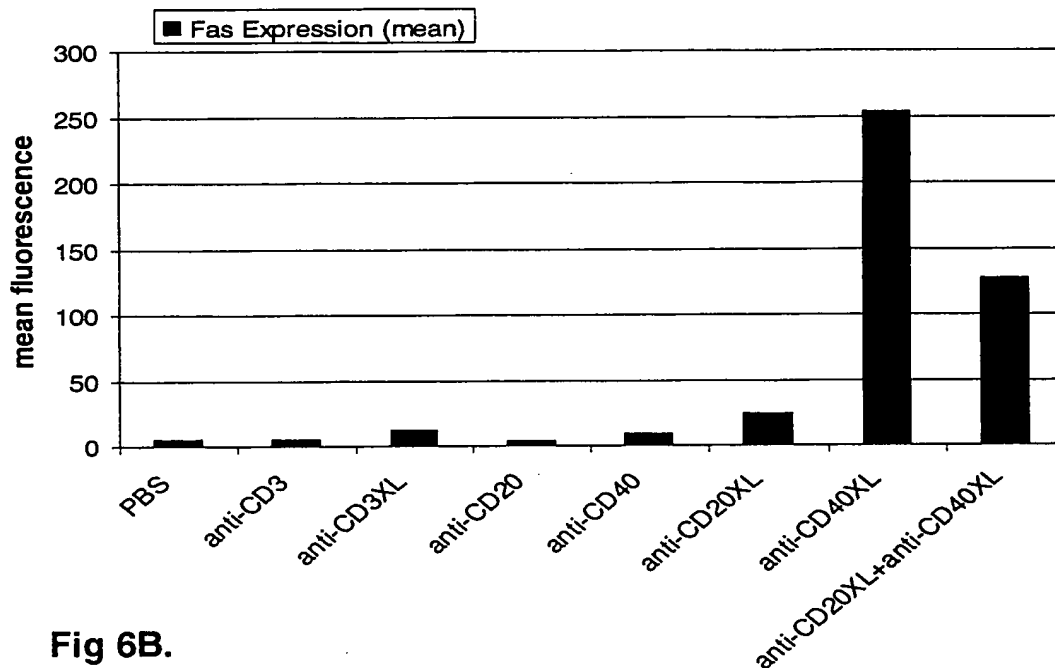


Fig 6B.

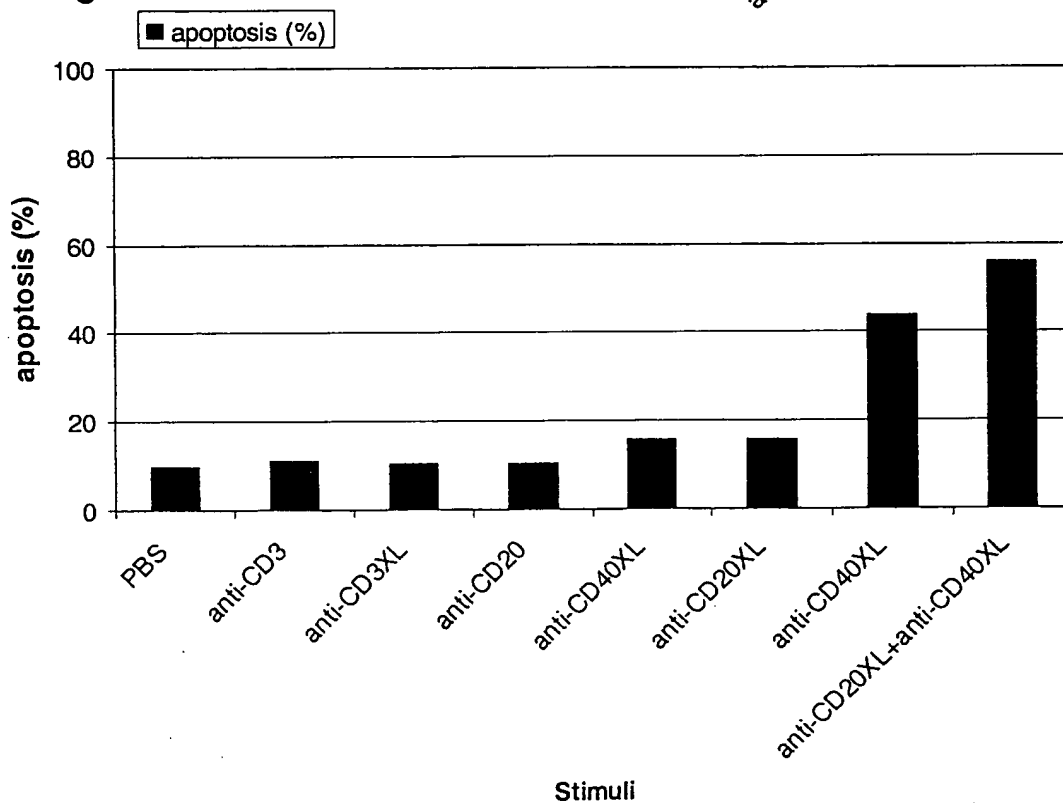


Fig. 7A

2H7-CD154 L2 cDNA and predicted amino acid sequence:

```

HindIII      NcoI  2H7 VL Leader Peptide →
-----
1  AAGCTTGCCG CC   M D F Q V Q I F S F L L I S A S
    ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                2H7 VL →
61  V I I A R G Q I V L S Q S P A I L S A S
    GTCATAATG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

    P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                BamHI
                                -----
181  Y Q Q K P G S S P K P W I Y A P S N L A
    TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

    S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTACAATC

    S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker →
361  P T F G A G T K L E L K G G G G S G G G
    CCCACGTTCT GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                2H7 VH →
421  G S G G G G S S Q A Y L Q Q S G A E L V
    GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

    R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

    N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

    P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

    V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

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## Fig. 7A (continued)

human CD154/amino acid 48→

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                                     Bcl/Bam hybrid site
781  G T G T T V T V S D P R R L D K I E D E
    GGCACAGGGA CCACGGTCAC CGTCTCTTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

    R N L H E D F V F M K T I Q R C N T G E
841  AGGAATCTTC ATGAAGATTT TGTATTTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

    R S L S L L N C E E I K S Q F E G F V K
901  AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

                                     BclI
961  D I M L N K E E T K K E N S F E M Q K G
    GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI
-----
1021 D Q N P Q I A A H V I S E A S S K T T S
    GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

    V L Q W A E K G Y Y T M S N N L V T L E
1081 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

    N G K Q L T V K R Q G L Y Y I Y A Q V T
1141 AATGGGAAAC AGCTGACCGT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                                     HindIII
                                     -----
1201 F C S N R E A S S Q A P F I A S L C L K
    TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

    S P G R F E R I L L R A A N T H S S A K
1261 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

    P C G Q Q S I H L G G V F E L Q P G A S
1321 CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

                                     NcoI
                                     -----
1381 V F V N V T D P S Q V S H G T G F T S F
    GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

                                     XhoI
                                     -----
                                     XbaI
                                     -----
1441 G L L K L E * * S R
    GGCTTACTCA AACTCGAGTG ATAATCTAGA
```

Fig. 7B.

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```
HindIII      NcoI
-----
-----2H7 VL Leader Peptide→
      M D F Q V Q I F S F L L I S A S
1  AAGCTTGCCG CC  ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61  GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121  CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                BamHI
                                -----
      Y Q Q K P G S S P K P W I Y A P S N L A
181  TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

      S G V P A R F S G S G S G T S Y S L T I
241  TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301  AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361  CCCACGTTTC GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                                2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421  GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481  AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541  AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

      P G N G D T S Y N Q K F K G K A T L T V
601  CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661  GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721  GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
```

Fig. 7B

human CD154/amino acid 108 →

BclI/Bam hybrid site

BclI  
G T G T T V T V S D P E N S F E M Q K G  
781 GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI  
-----  
D Q N P Q I A A H V I S E A S S K T T S  
841 GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

V L Q W A E K G Y Y T M S N N L V T L E  
901 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTTGAA

N G K Q L T V K R Q G L Y Y I Y A Q V T  
961 AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII  
-----  
F C S N R E A S S Q A P F I A S L C L K  
1021 TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

S P G R F E R I L L R A A N T H S S A K  
1081 TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

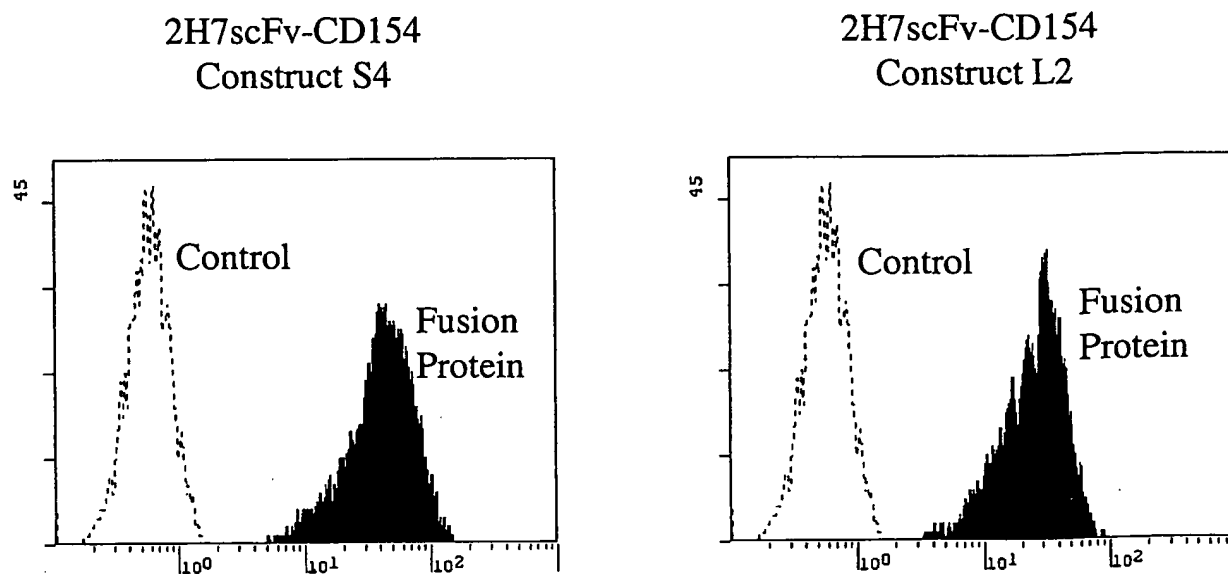
P C G Q Q S I H L G G V F E L Q P G A S  
1141 CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI  
-----  
V F V N V T D P S Q V S H G T G F T S F  
1201 GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI XbaI  
-----  
G L L K L E \* \* S R  
1261 GGCTTACTCA AACTCGAGTG ATAATCTAGA

Fig. 8

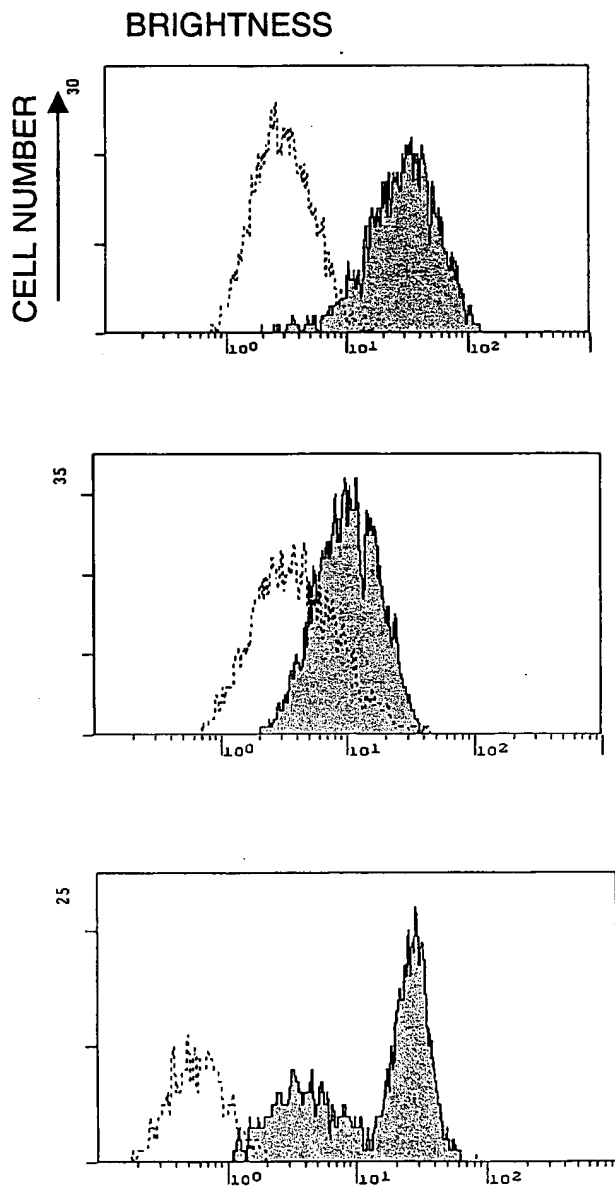
Simultaneous Binding of 2H7scFv-CD154  
Fusion Proteins to CD20 and CD40



CD20 CHO cell targets + (control or fusion protein)  
+ Biotin-CD40Ig + PE-SA

Fig. 9

Induction of Apoptosis Measured by Binding of Annexin V after  
incubation with 2H7scFv-CD154



.....control supernatant    \_\_\_\_2H7scFv-CD154 supernatant

Fig. 10

Proliferation of T51 B Cell Line After Incubation with 2H7  
scFv-CD154 S4 or 2H7 scFv-CD154 L2 Constructs

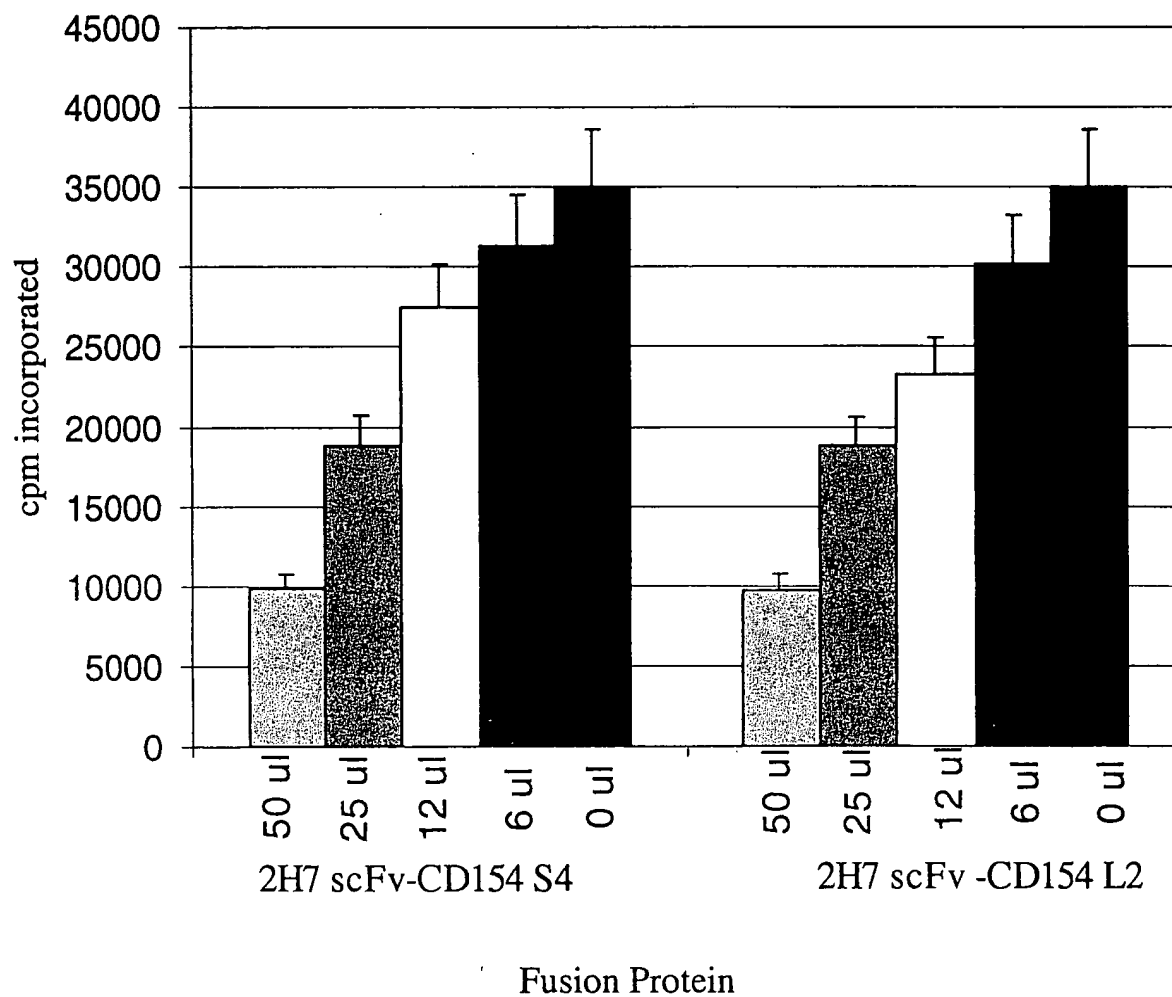




Fig. 11

## Schematic Representation of 2H7 scFvIg Constructs

2H7 scFvIgG (SSS-S)H WCH2 WCH3

OR 2H7 scFvIgG1 (SSS-S)H P238SCH2 WCH3 : 2H7 scFv

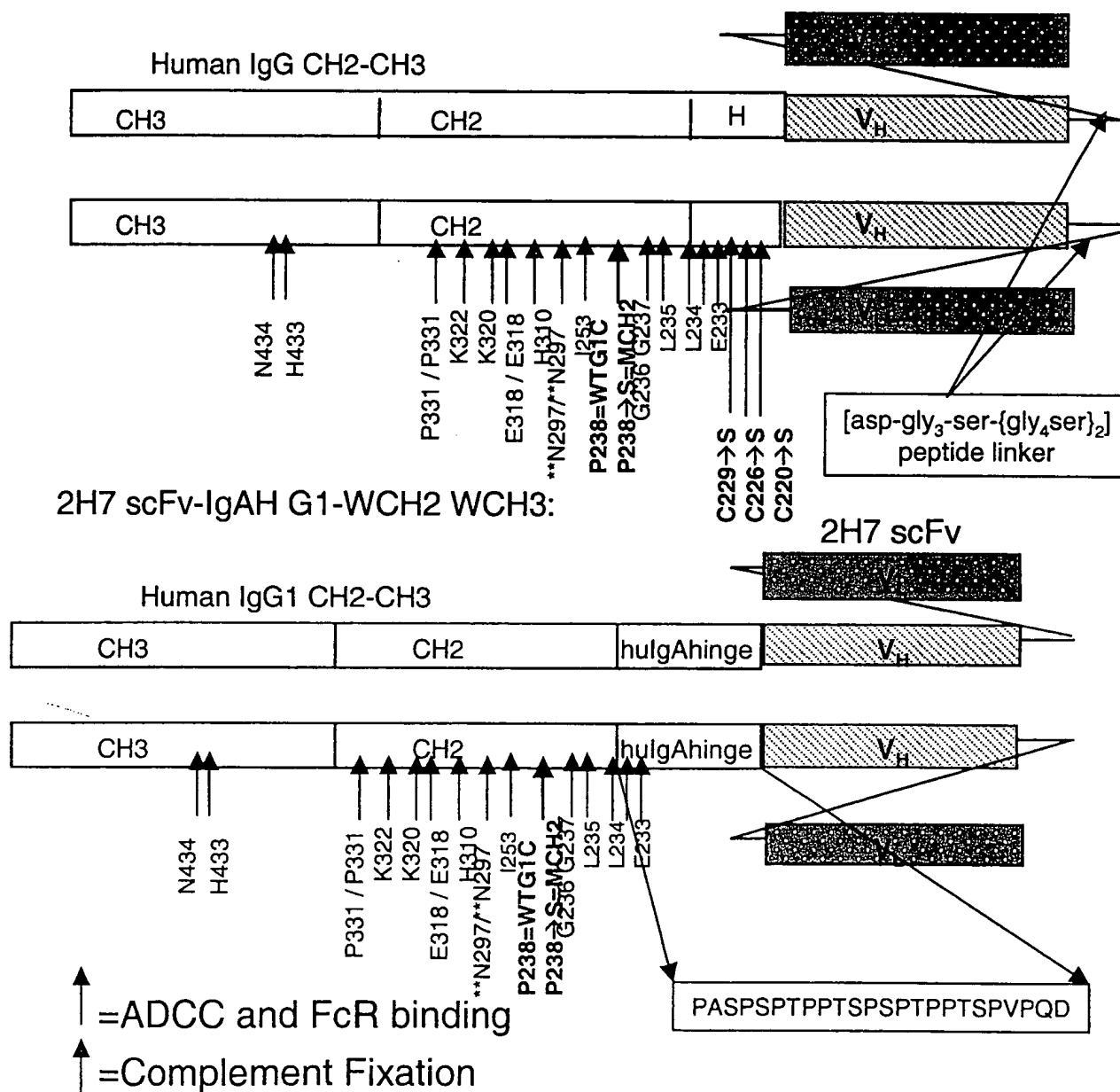


Fig. 12

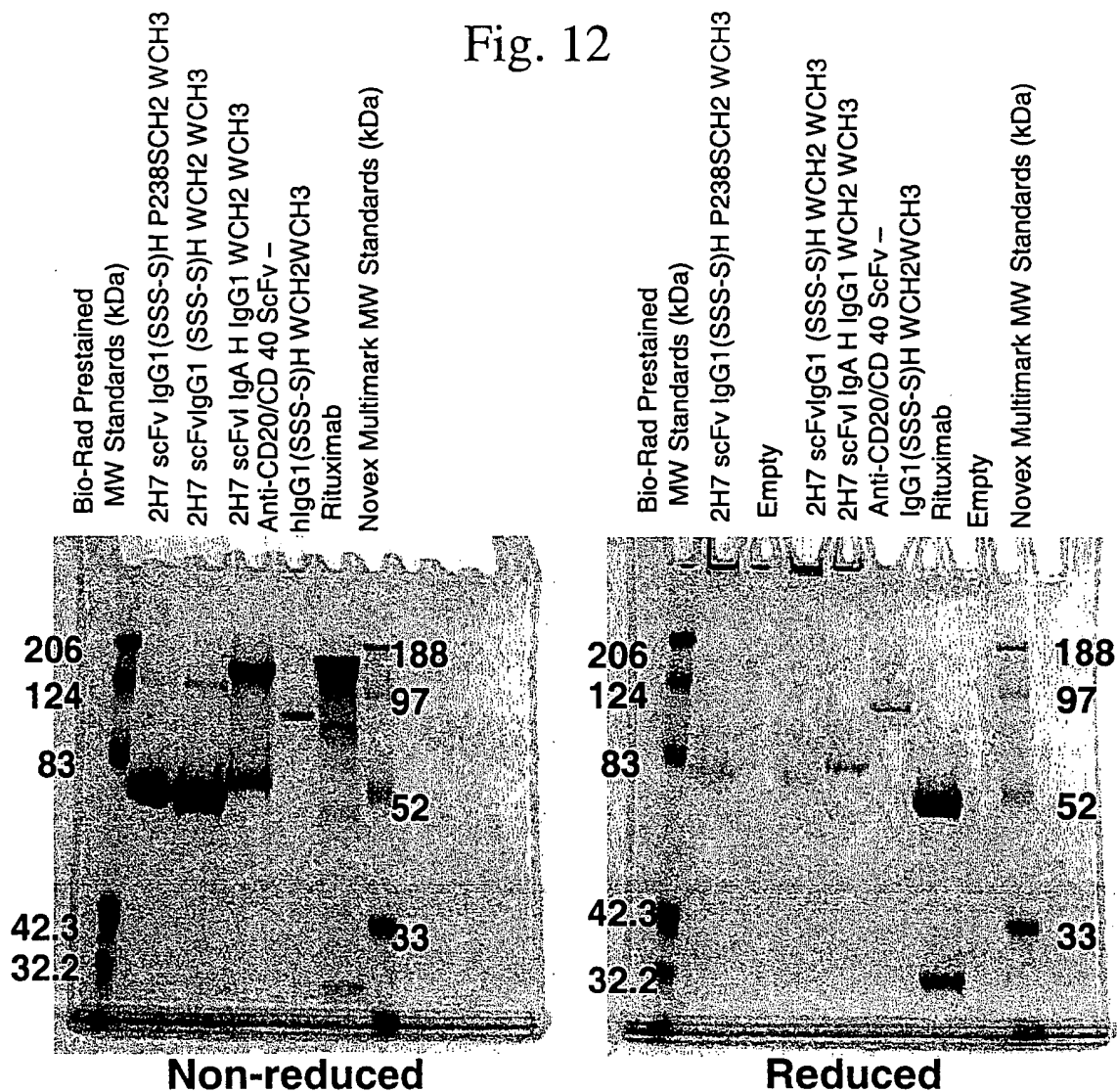


Figure 12: SDS-PAGE Analysis of CytoxB Derivatives. Purified fusion protein derivatives of CytoxB-scFvIg molecules and Rituximab were resuspended in SDS sample buffer, boiled, loaded onto 10% Novex Tris-Bis gels (Invitrogen, San Diego, CA) and subjected to nonreducing (left panel) or reducing (right panel) SDS-PAGE electrophoresis at 175 volts. Two different molecular weight markers, BioRad prestained markers, and Novex Multimark molecular weight markers were also loaded onto each gel and the approximate size in kDa of each marker band is indicated along each side of the photographed gels. Gels were stained in Coomassie Blue stain and photographed with a SONY Mavica Digital camera. The mutant hinge forms of 2H7 scFvIgG1 migrate at approximately 70 kDa under both nonreducing and reducing conditions, indicating that these molecules are monomeric rather than dimeric in structure. The IgA hinge form of 2H7scFvIg migrates at approximately 75 kDa under reducing conditions, but migrates predominately as a dimer of 140 kDa with a fraction of the protein migrating at 75 kDa under nonreducing conditions. Under nonreducing conditions, rituximab migrates as a diffuse band of between 150 and 200 kDa. The heavy and light chains resolve into separate bands of approximately 32 and 50 kDa when rituximab is reduced and subjected to SDS-PAGE.

Fig. 13  
ADCC Activity of Cytox B (2H7 scFvIg) Constructs.

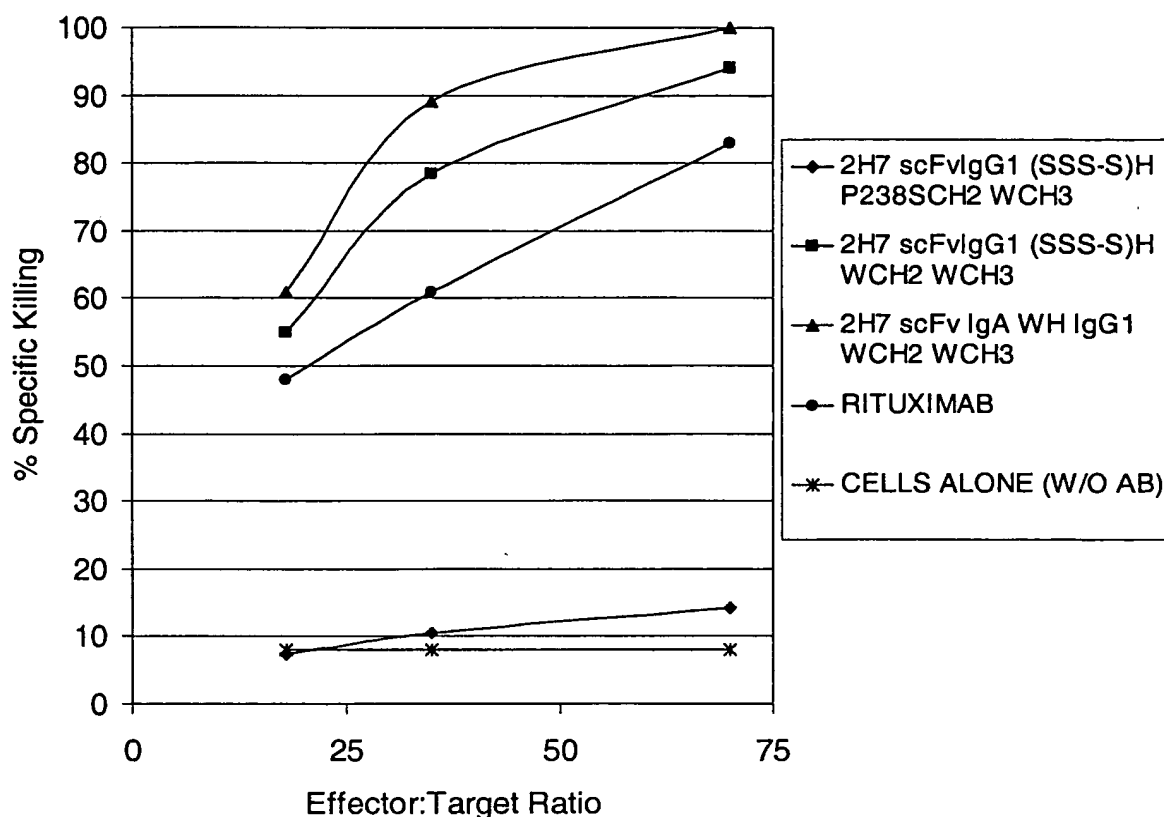


Figure 13: ADCC Activity of Cytox B Derivatives Compared to Rituximab. ADCC activity of Cytox B Derivatives or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. Bjab cells were labeled for 2 hours with  $^{51}\text{Cr}$  and aliquoted at a cell density of  $5 \times 10^4$  cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 mg/ml, and PBMC were added at  $9 \times 10^5$  cells/well (18:1),  $1.8 \times 10^6$  cells/well (35:1), or  $3.6 \times 10^6$  cells/well (70:1), in a final volume of 200  $\mu\text{l}$ . Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100  $\mu\text{l}$  culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

Fig. 14  
CDC of Cytos B (2H7 scFvIg) Constructs

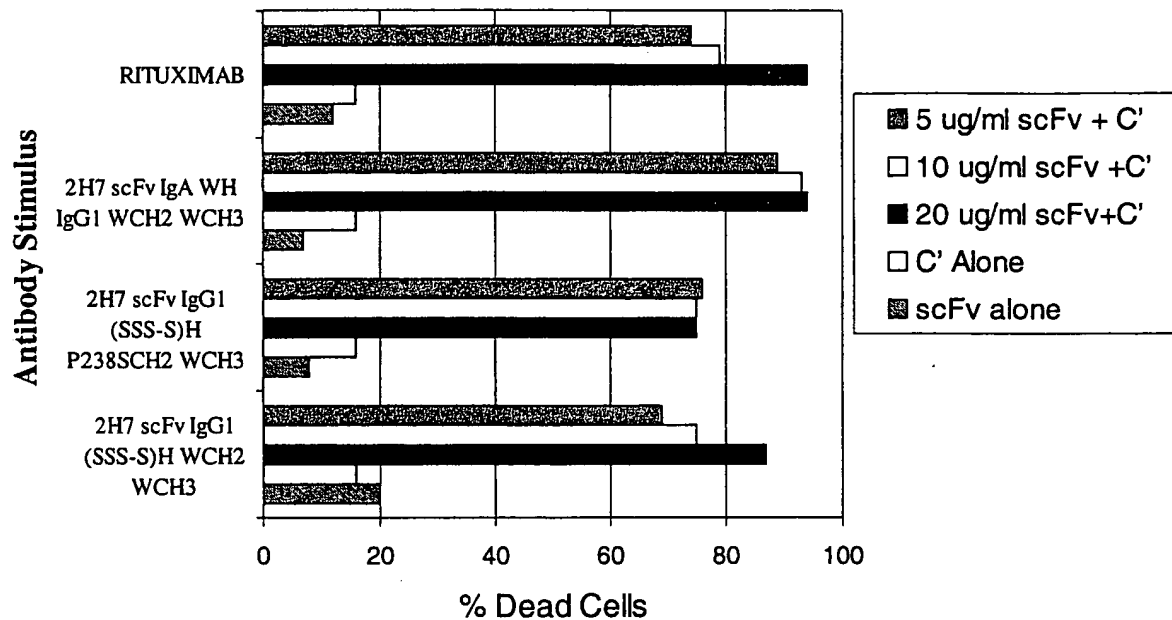
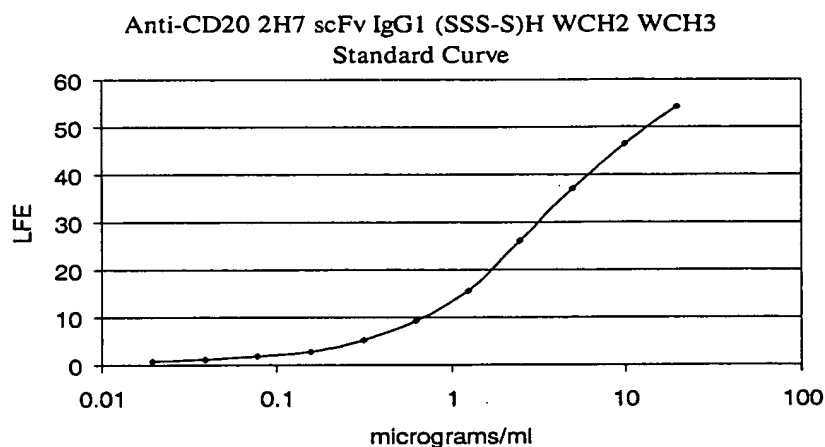


Figure 14: Complement Dependent Cytotoxicity (CDC) Activity of CytosB Derivatives Compared to Rituximab. 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, and 2H7scFv IgA WH IgG1 WCH2 WCH3 derivatives and Rituximab were compared for their ability to mediate complement dependent cytotoxicity. Rabbit complement (Pel-Freez) was diluted 1:10 and added to BJAB cells along with dilutions of each antibody derivative (20  $\mu$ g/ml, 10  $\mu$ g/ml, and 5  $\mu$ g/ml). Controls were also included without addition of complement (C') or scFv derivative. Reactions were allowed to continue for 1 hour, and cells from each well were then stained with trypan blue and the cell viability counted using a hemacytometer. Data is graphed as % of dead cells/total cells counted for each condition assayed.

Fig. 15  
2H7 (anti-CD20) scFv IgG1 (SSS-S)H WCH2 WCH3  
In Vivo Half Life



Macaque A99314

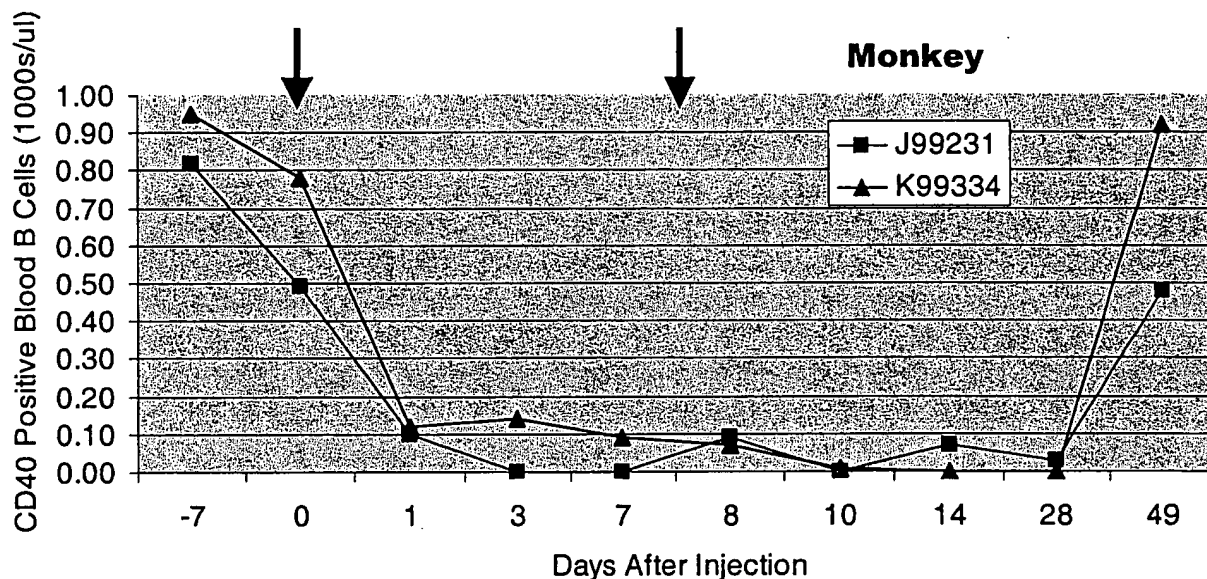
	Day	Binding intensity (LFE) @ 1:50 dilution of serum	estimated concentration (µg/ml)
Injection #1	-7	0.213	<0.1
	0	0.227	<0.1
	1	7.79	25.1
Injection #2	3	5.51	15.6
	7	3.37	9.4
	8	11.33	41.7
	10	5.45	15.4
	14	0.27	<0.1

Macaque F98081

	Day	Binding intensity (LFE) @ 1:50 dilution of serum	estimated concentration (µg/ml)
Injection #1	-7	0.208	<0.1
	0	0.219	<0.1
	1	6.73	21.9
Injection #2	3	6.14	19.3
	7	3.04	8.7
	8	9.83	33.8
	10	4.77	14.4
	14	0.231	<0.1

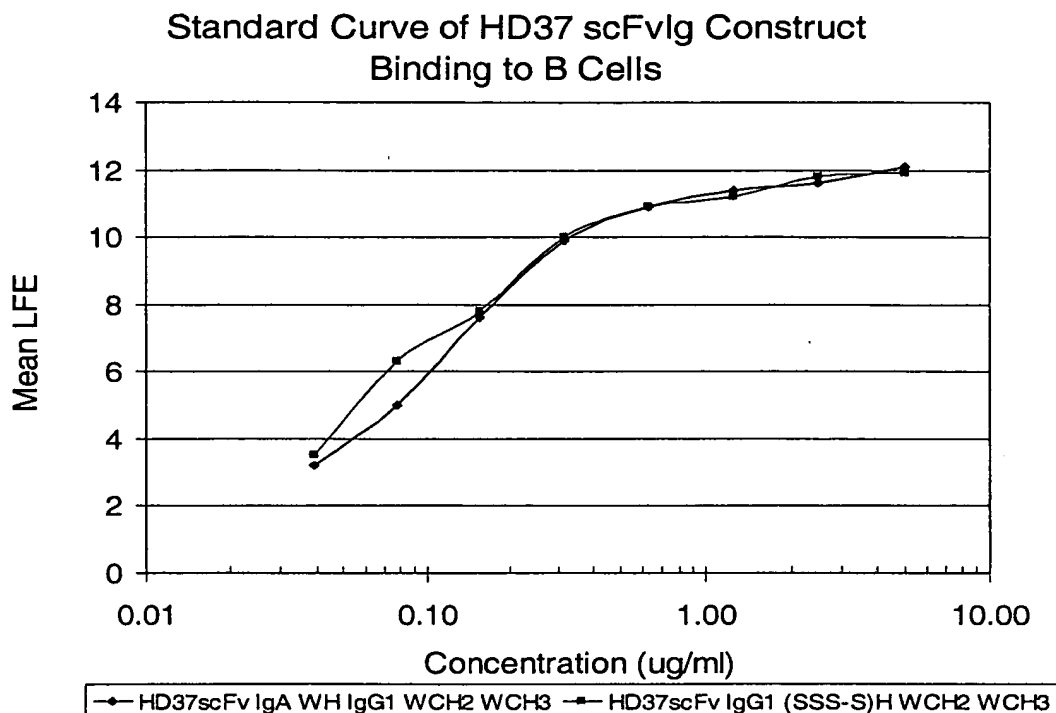
Fig. 16

B Cell Depletion in macaques mediated by Cytos B20  
(2H7 scFv IgG1 (SSS-S)H WCH2 WCH3) Construct



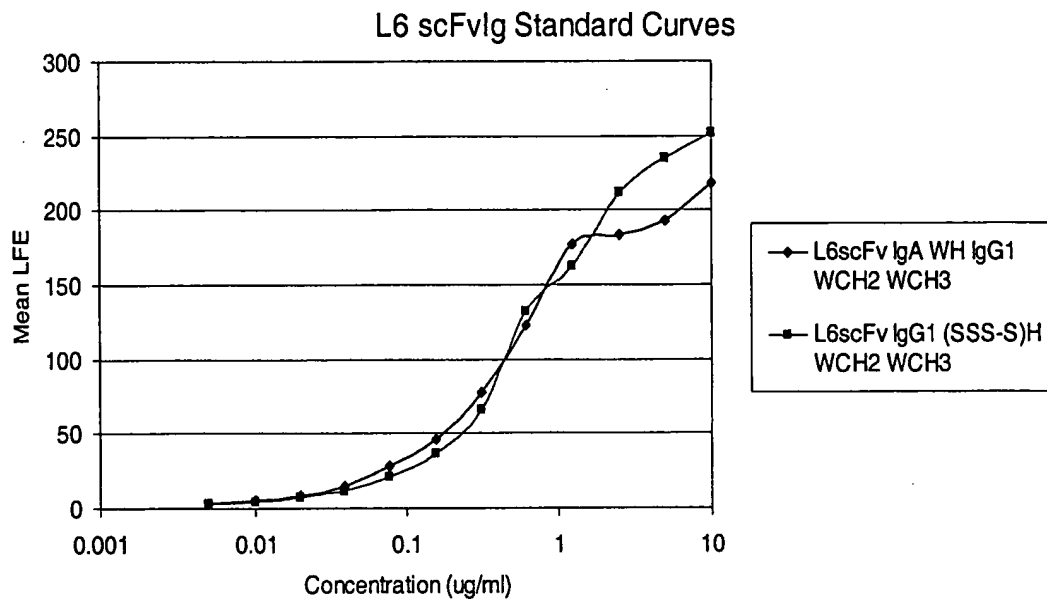
- CytosB20 injections of 6mg/kg yields 3 week B-cell depletion
- 3-4 day half-life *in vivo*
- CD20 saturation in lymph node B-cells at d14
- No first dose effects
- No anti-chimeric antibody development

Fig. 17  
Production Levels of HD37 scFvIg Constructs  
by CHO Cell Lines



Clone/Isolate	Mean LFE at 1:100	Estimated Concentration
Bulk HD37 scFv		
IgA WH IgG1 WCH2 WCH3	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
Bulk HD37 scFv		
IgG1 (SSS-S)H WCH2 WCH3	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

Fig. 18  
Production of L6 scFvIg constructs by CHO Cells



Construct	Mean LFE 1:20	Estimated Concentration
L6scFv IgA WH IgG1 WCH2 WCH3 unamplified CHO sup	51.1	6.25 ug/ml
L6scFv IgG1(SSS-S)H WCH2 WCH3 unamplified CHO sup	23.0	3.2ug/ml

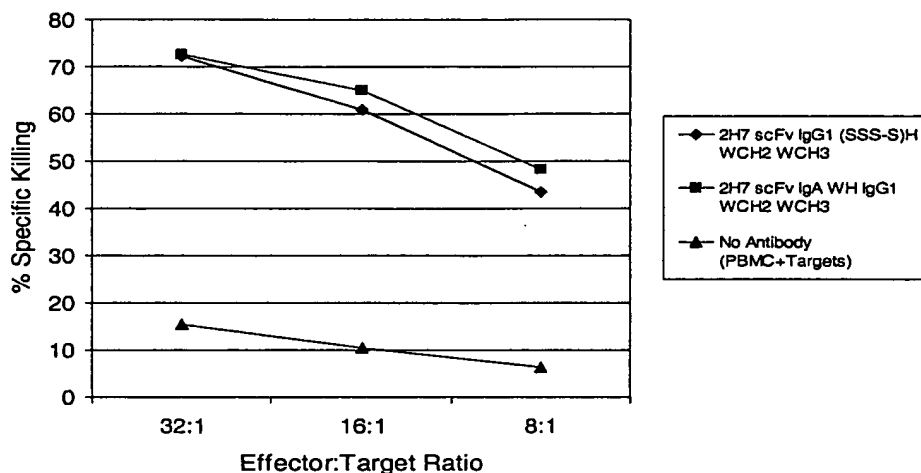


# Fig. 19

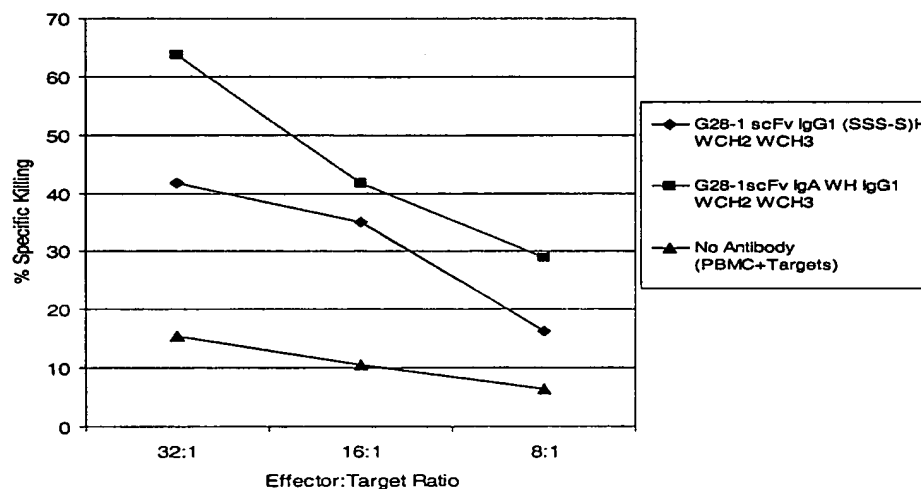
## ADCC Activity of 2H7 scFvIg, G28-1 scFvIg, and HD37 scFvIg Constructs

### ADCC Activity of scFvs Targeted to B Cell Antigens

A. 2H7 (anti-CD20) scFv constructs



B. G28-1 (anti-CD37) scFv constructs



C. HD37 (anti-CD19) scFv constructs

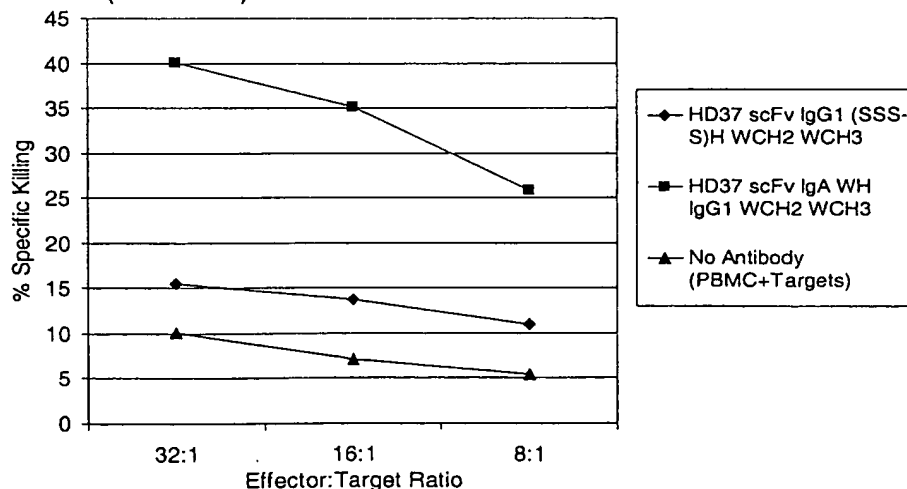


Fig. 20  
ADCC Activity of L6 scFvIg Constructs

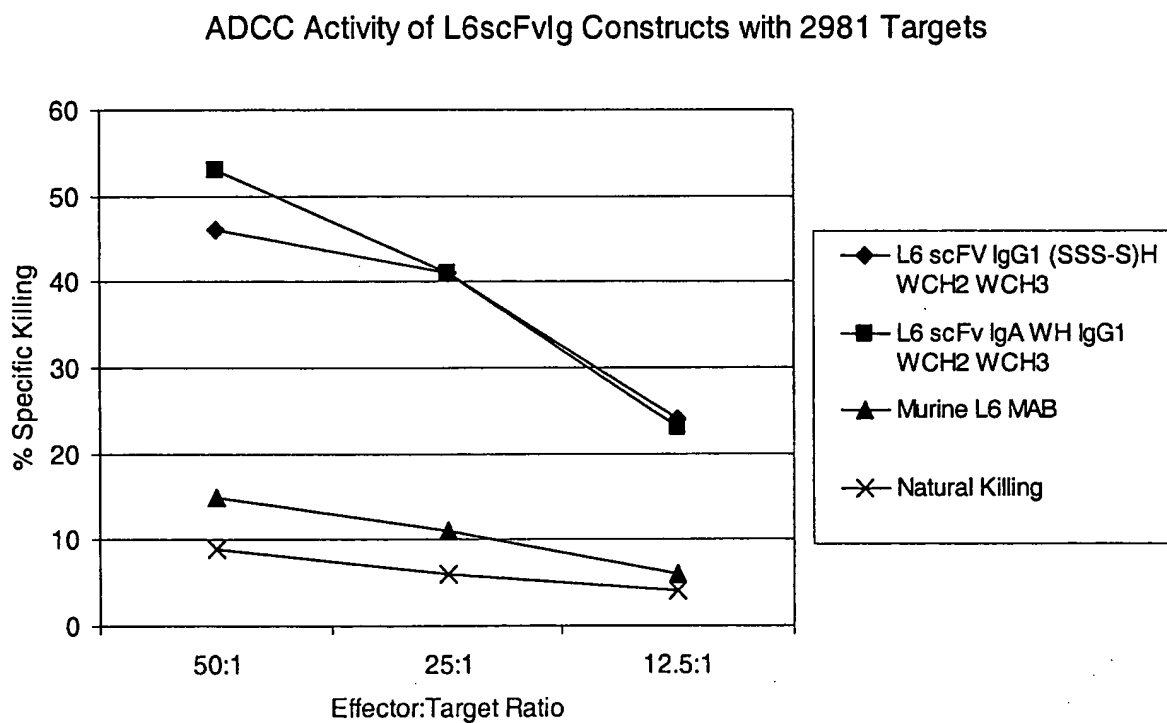


Fig. 21  
SDS-PAGE Analysis of L6 and 2H7  
scFvIg Fusion Proteins.

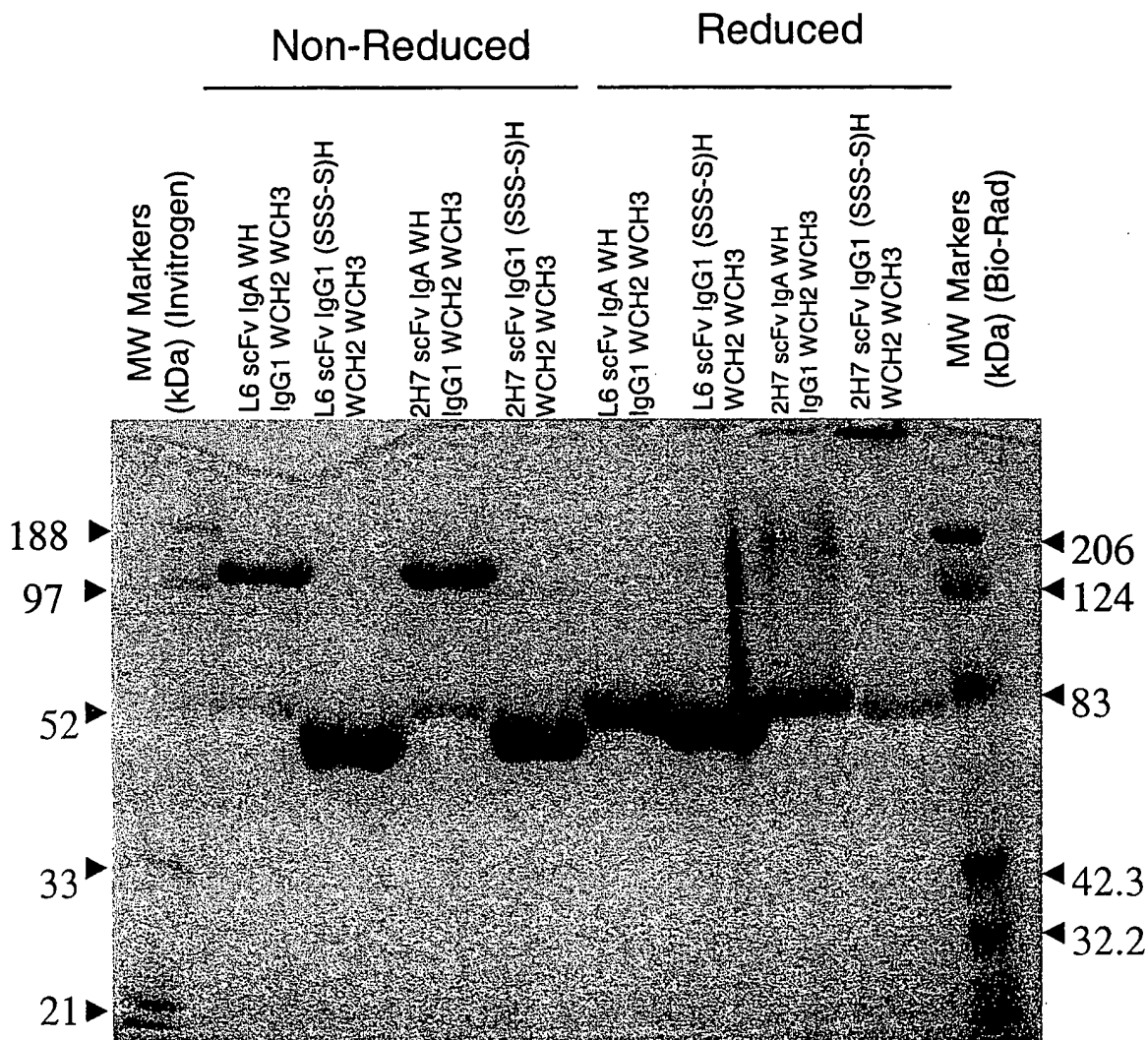


Fig. 22  
SDS-PAGE Analysis of G28-1 and HD37  
scFvlg Constructs.

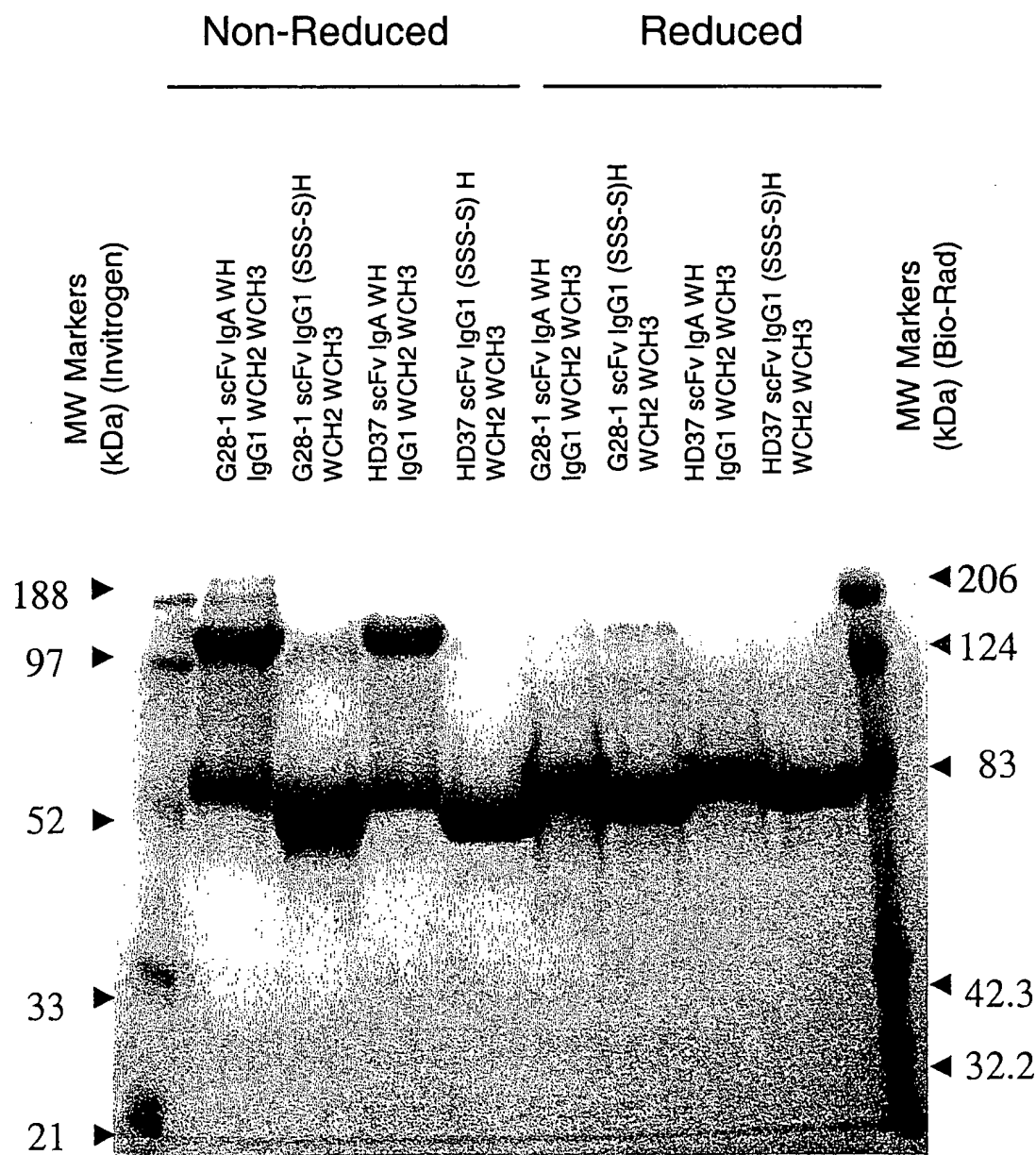


Fig. 23  
Sequence alignment of human and llama Fc regions.

	HINGE	CH2→
Human IgG1:	DQEPKSCDKT-----HTCPPC	PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
Llama IgG2:	DQEPKTPKPQPQPQPQPNPTTESKCPKC	PAPELLGGPSVFIFFPKPKDVLISIGRPEVTCVVVDVGQEDPEVSFNWYIDG
Llama IgG1:	--EPHGG-----CTCPQC	PAPELPGGPSVFVFPPKPKDVLISIGRPEVTCVVVDVGKEDPEVNFNWIYIDG
Llama IgG3:	--AHHSEDPT-----SKCPKC	PGPELLGGPTVFIFPPKAKDVLISITRKPEVTCVWWTWVKKTLRSSSSWSVDD

VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT  
TAEVRANTRPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIEKTISKAKGQTPREPQVYTLAPHREELAKDTVSVT  
VEVRTANTKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYTLAPHREELAKDTVSVT  
TEVHTAETKPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYTLAPHREELAKDTVSVT

CLVKGFYPSDIAVEWESNGQPEN--NYKTTTPFVLDSGDSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK  
CLVKGFYPDPINVEWQRNGQPESXGTYATTPQLDNDGTYFLXSKXSVGKNTWQQGETFTCVVMHEALHNHYTQKSITQSSGK  
CLVKGFYPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSLVSGKNTWQRGETLTGVVMHEALHNHYTQKSITQSSGK  
CLVKGFFPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSLVSGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

Fig. 24

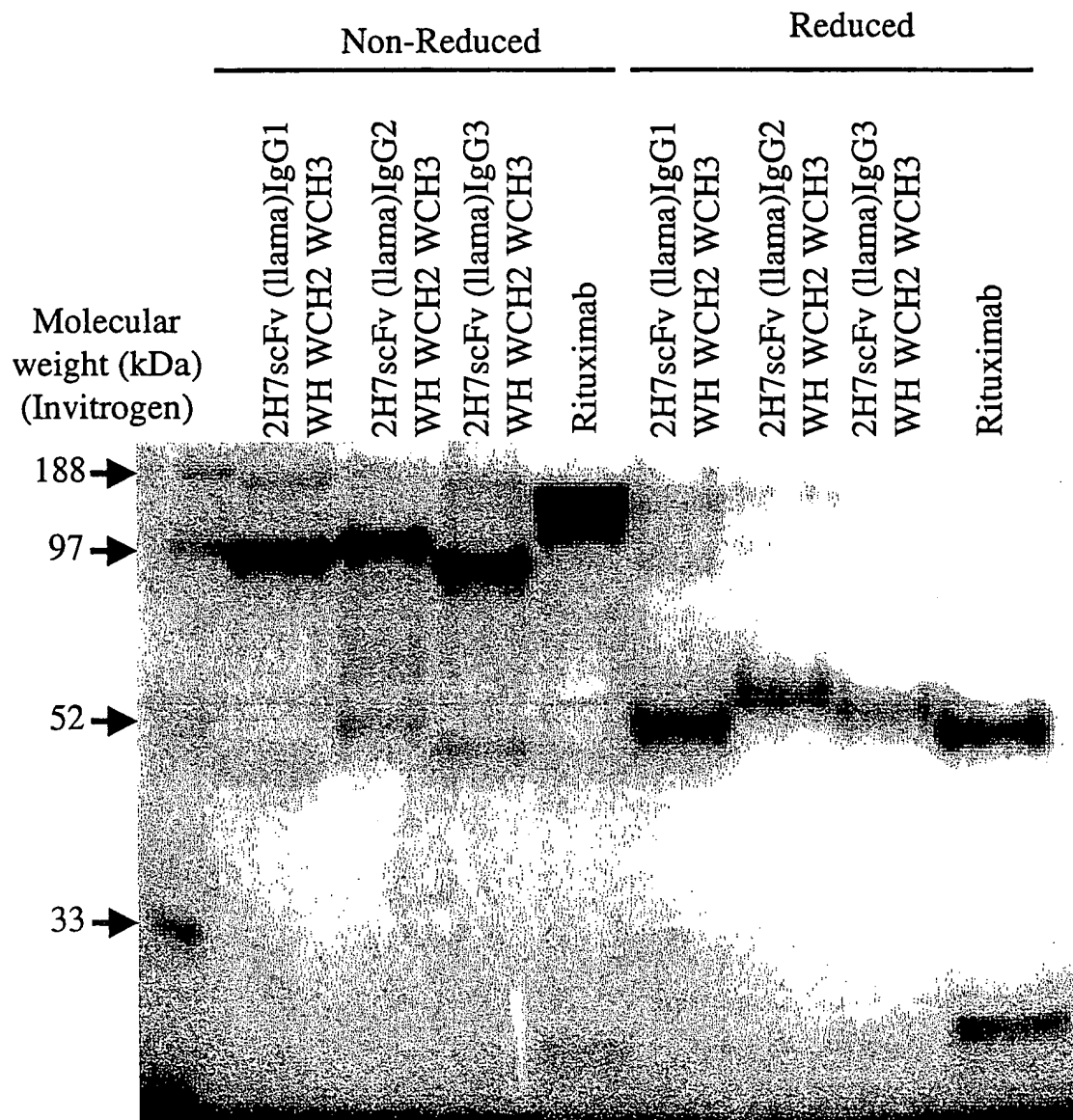


Fig. 25

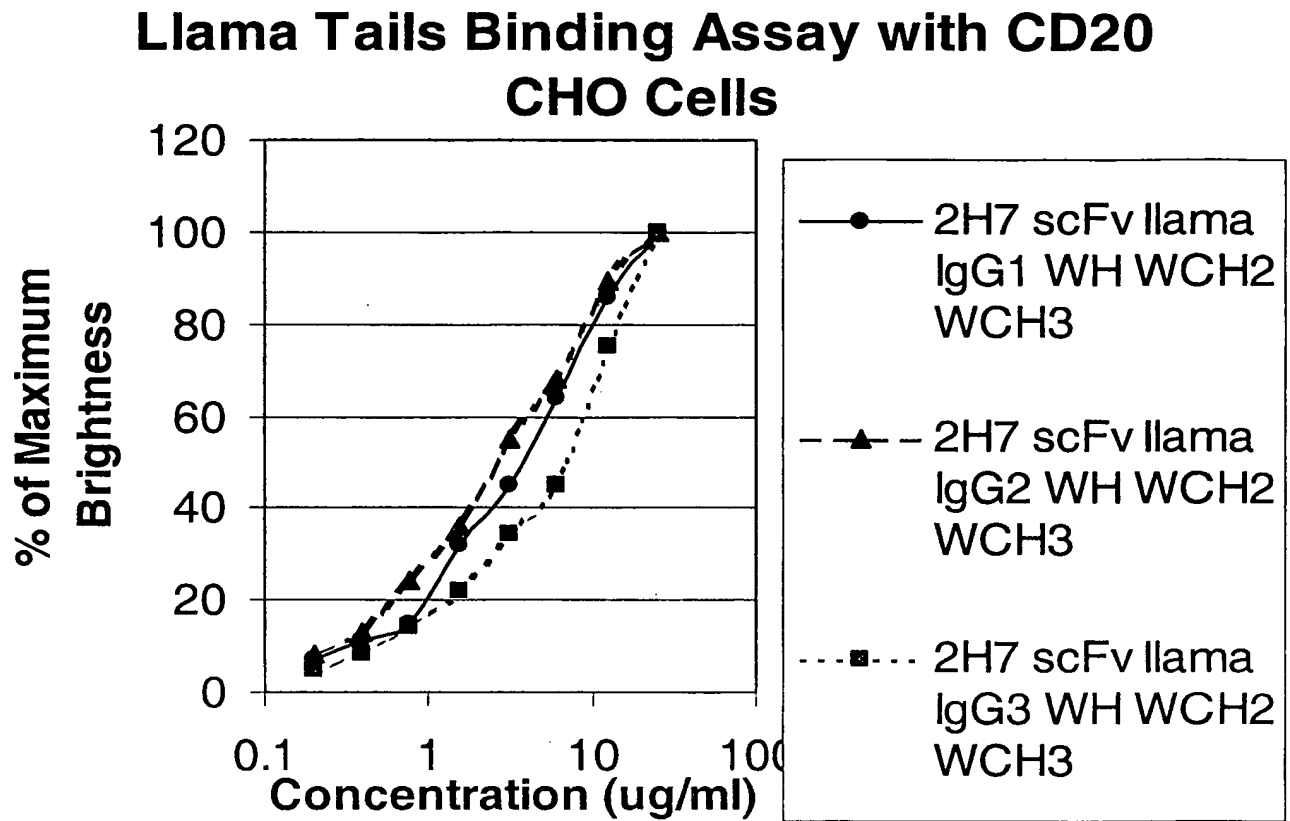
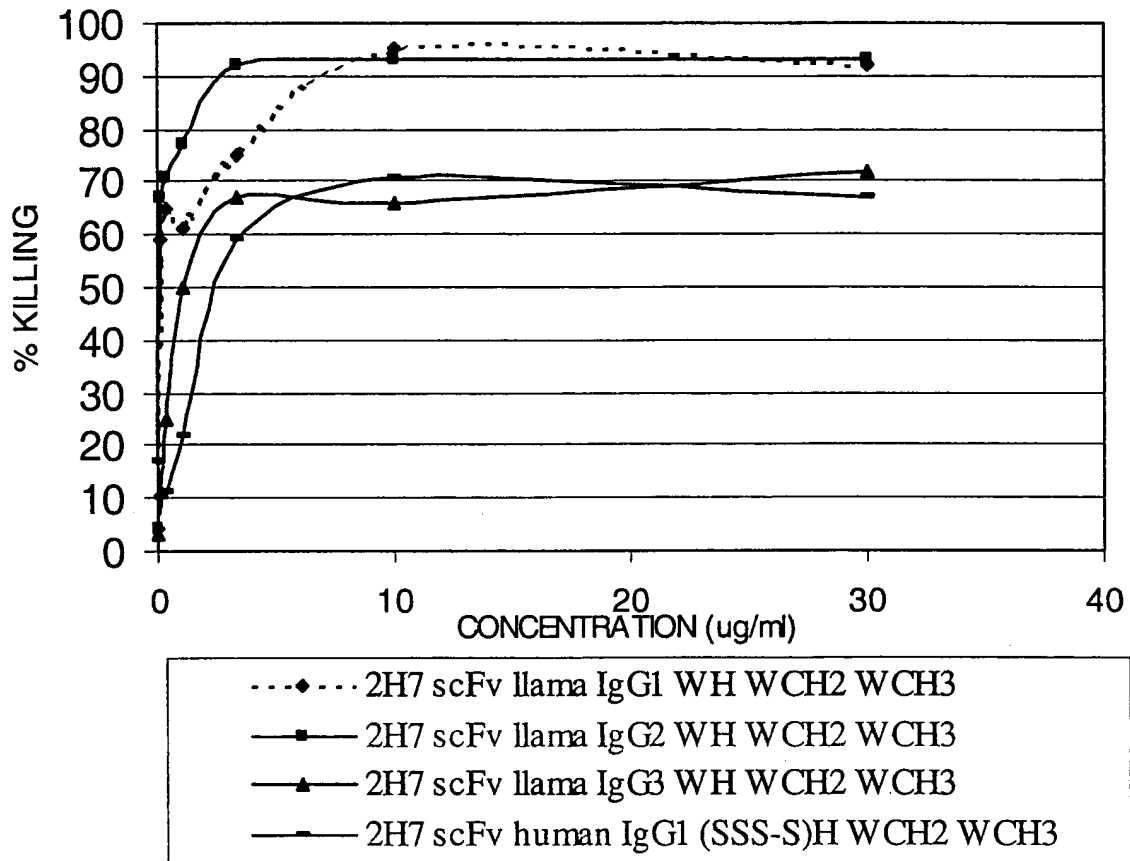


Fig. 26

2H7 scFvIg Llama Tails binding Assay with CD20 CHO Cells





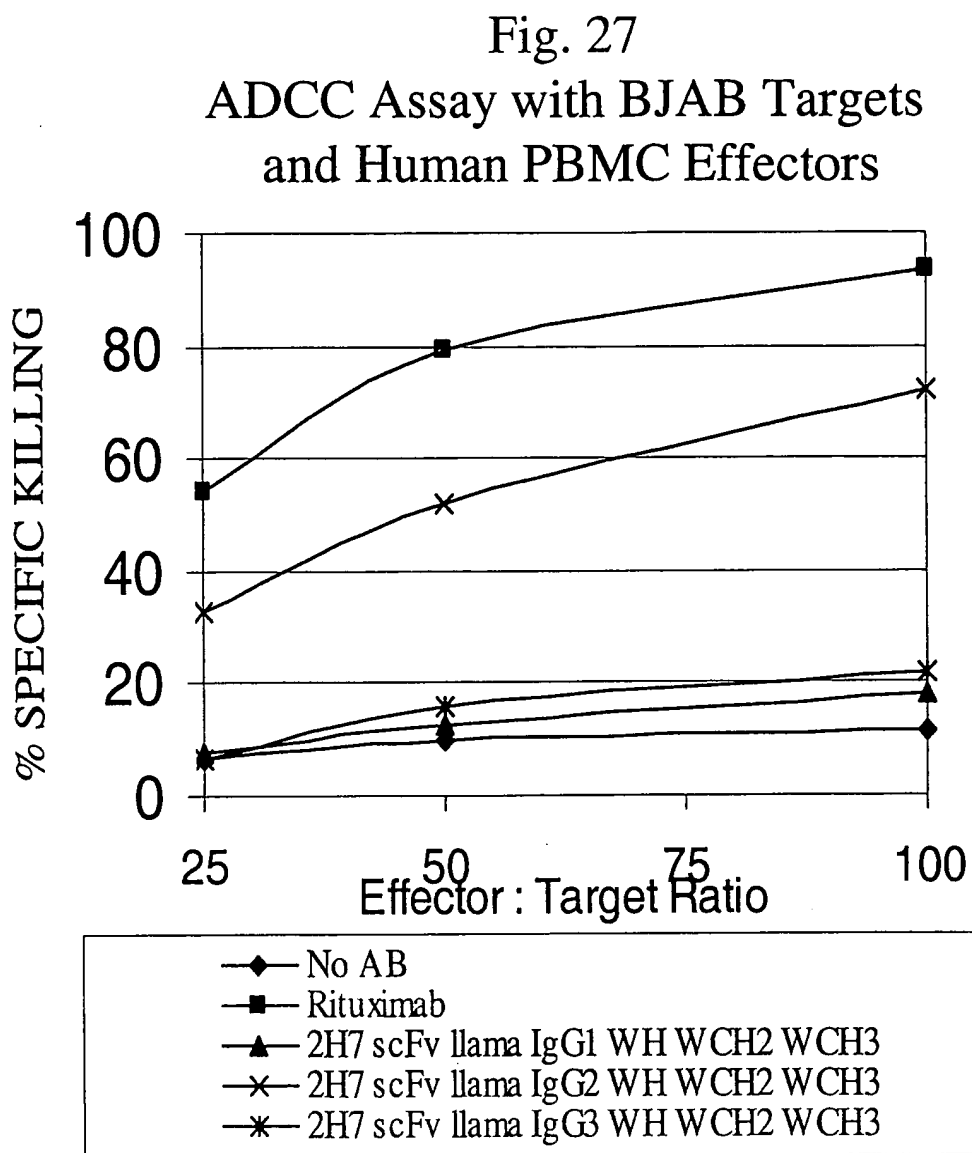


Fig. 28

ADCC Assay with BJAB Cells  
And Llama PBMC Effectors

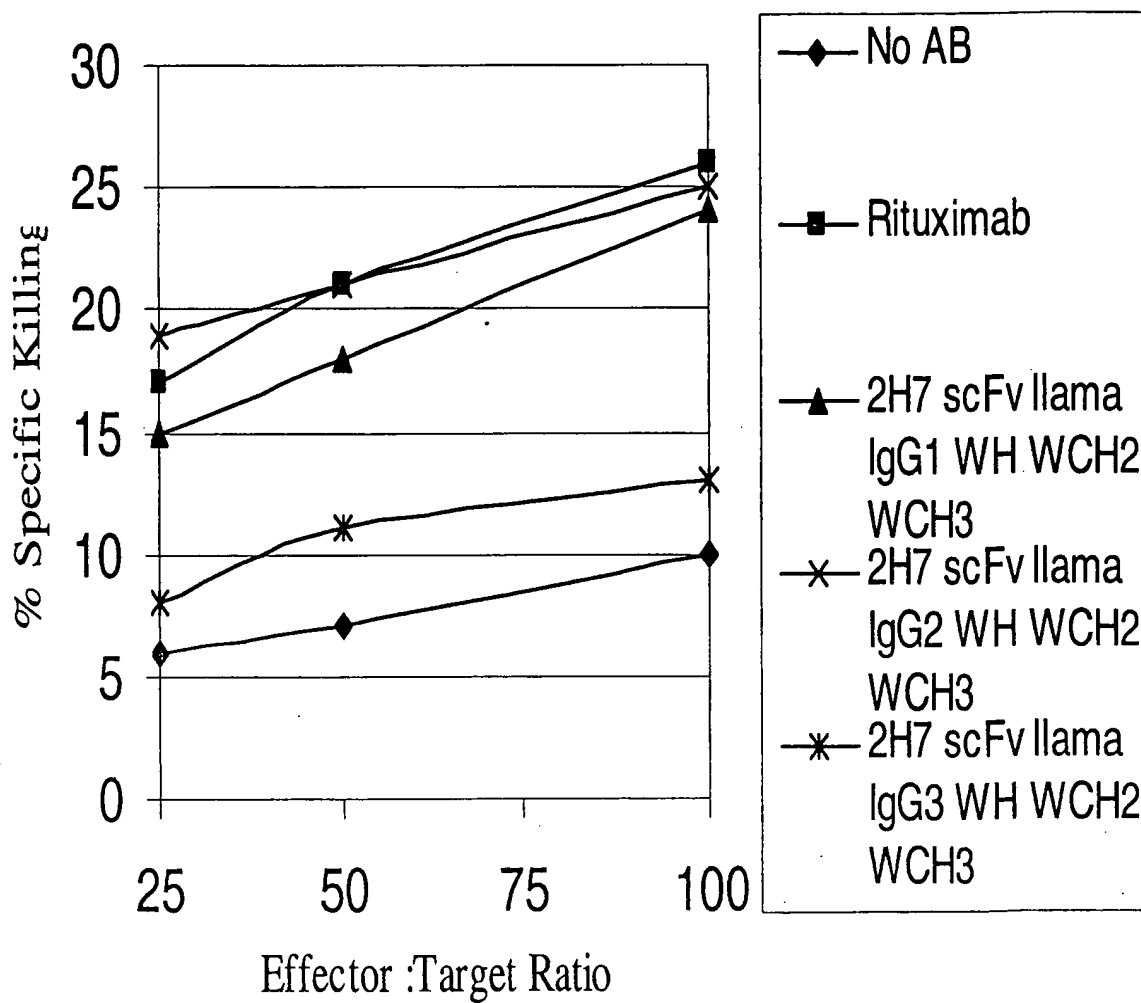


Fig. 29

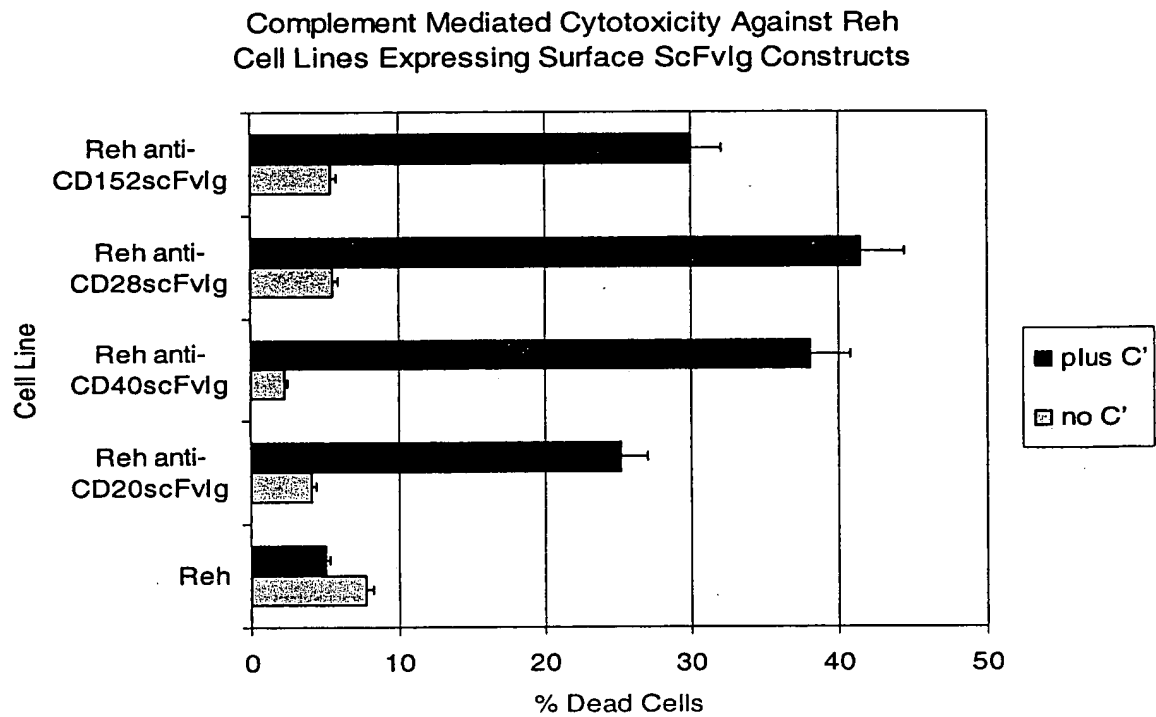


Fig. 30

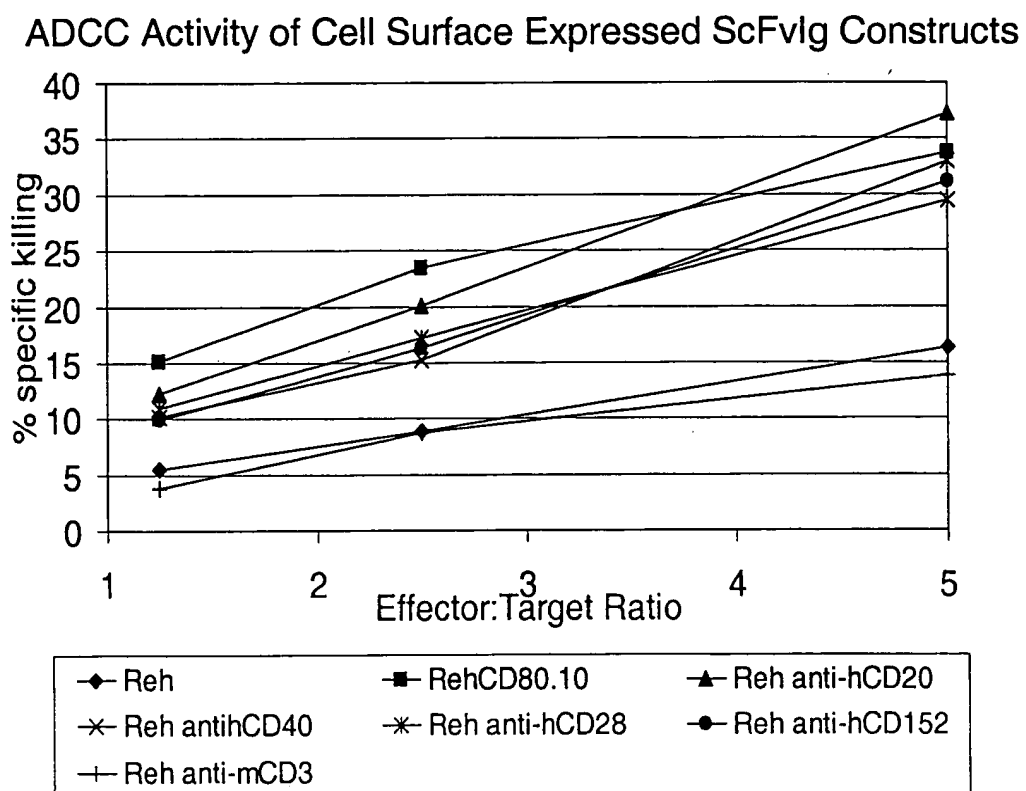


Fig. 31  
Ig Constructs and Nomenclature:

Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence
hIgG1 (CCC-P)H WCH2 WCH3	IgG1 WT Hinge (CCC-P)	Wild Type CH2	Wild Type CH3
hIgG1 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
VH L11S hIgG1 (SSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SSC-S)H WCH2 WCH3	IgG1 Mutant Hinge (SSC-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SCS-S)H WCH2 WCH3	IgG1 Mutant Hinge (SCS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (CSS-S)H WCH2 WCH3	IgG1 Mutant Hinge (CSS-S)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgG1 (SSS-S)H P238S CH2 WCH3	IgG1 Mutant Hinge (SSS-S)	Mutant CH2 (IgG1) Pro→Ser 238	Wild type CH3 (IgG1)
IgA WH hIgG1 WCH2 WCH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgA WH IgA WCH2 WCH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)
IgA WH IgA WCH2 T4CH3	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) Missing 4 aa at COOH

Fig. 32

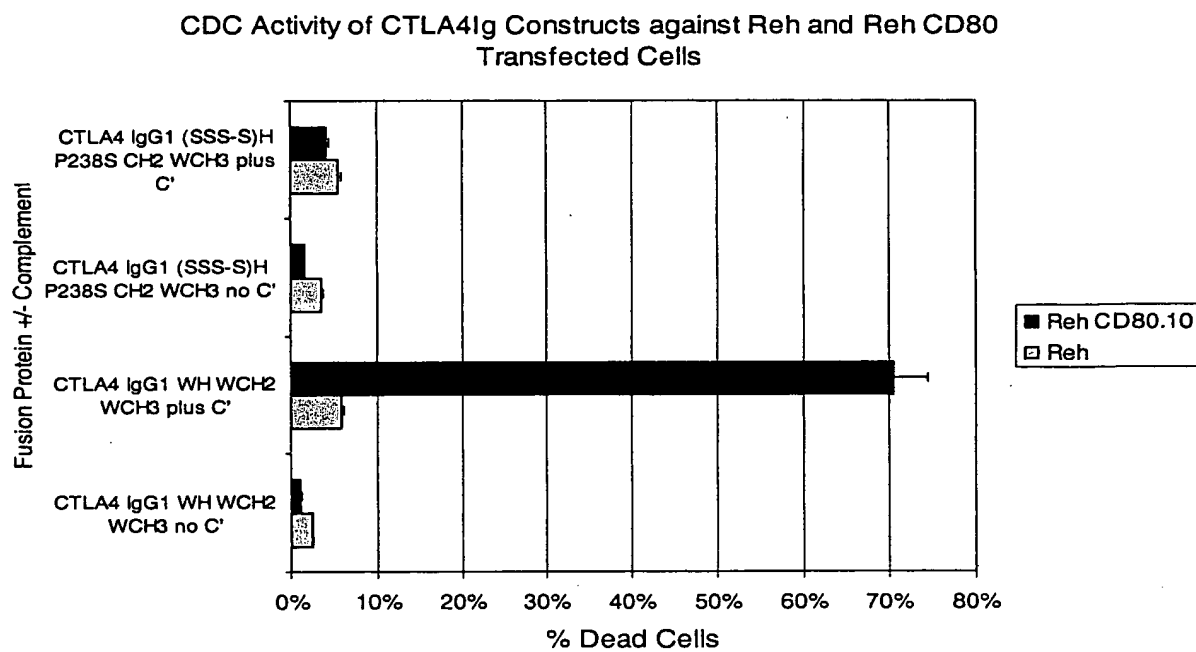


Fig. 33

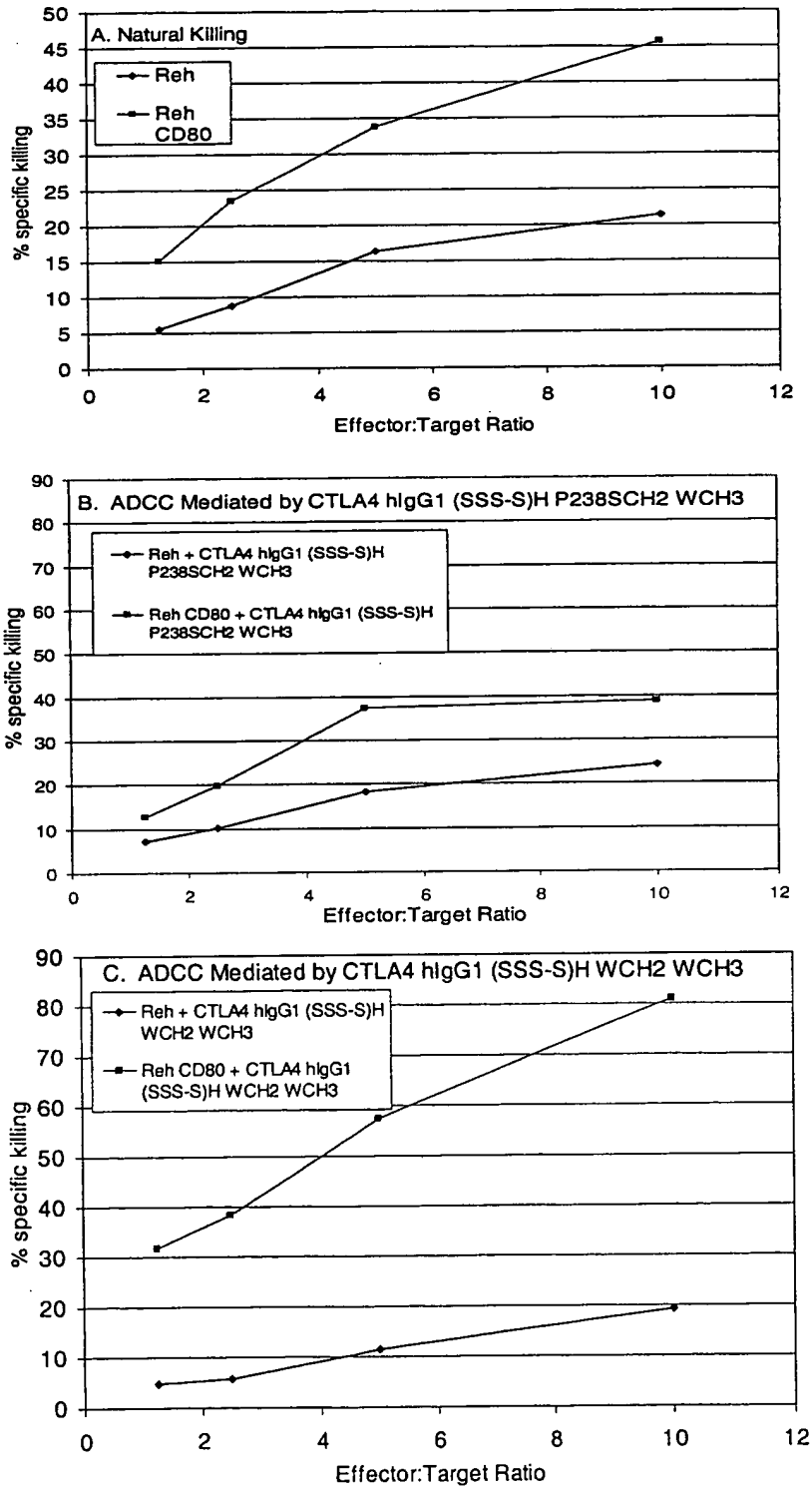


Fig. 34

Binding of 2H7 scFvIg Constructs  
with Alternative Tails to CD20 CHO Cells

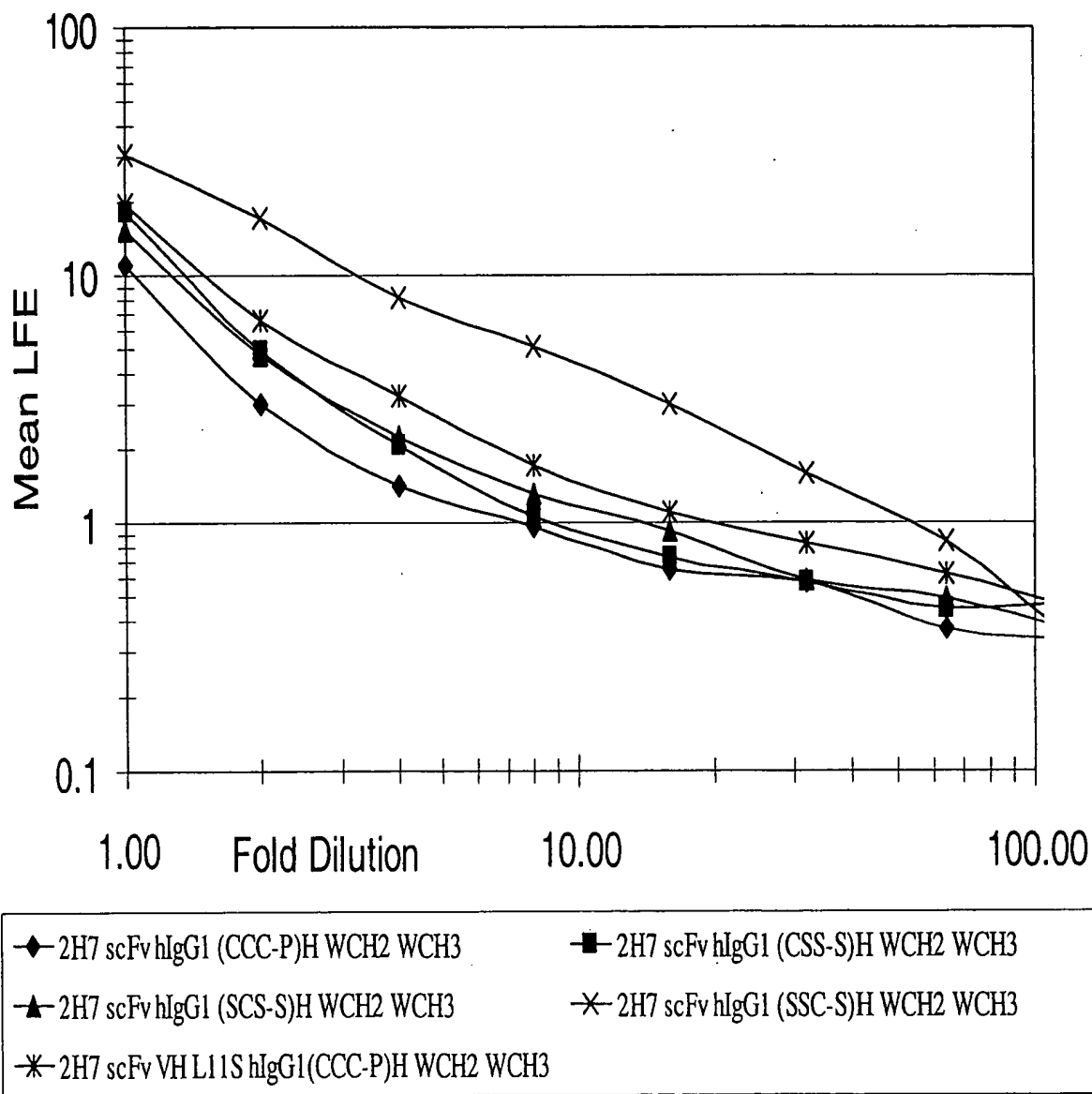




Fig. 35  
Immunoblot Analysis of protein immunoprecipitates  
from COS transfections of 2H7 scFvIg Constructs

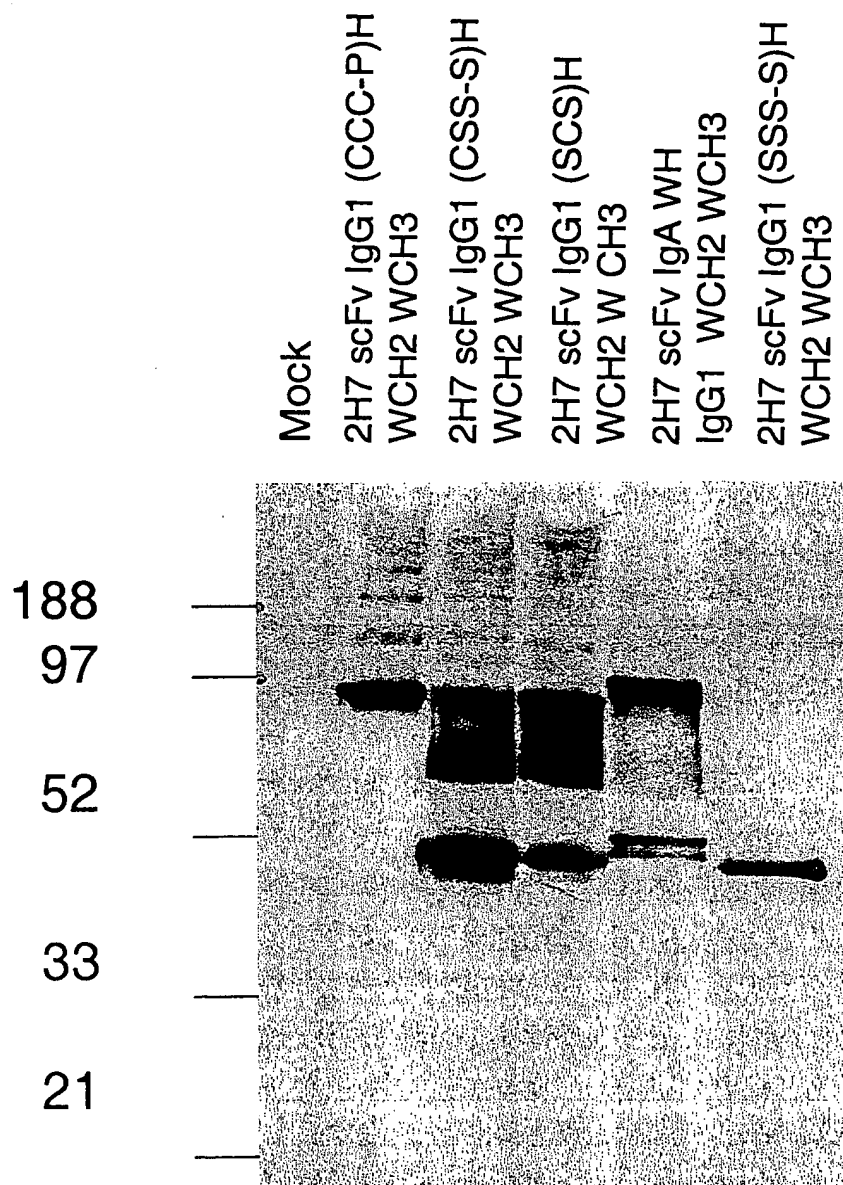


Fig. 36

Binding to CD20 CHO cells by constructs  
that link anti-CD20 scFv to IgA Fc Domains

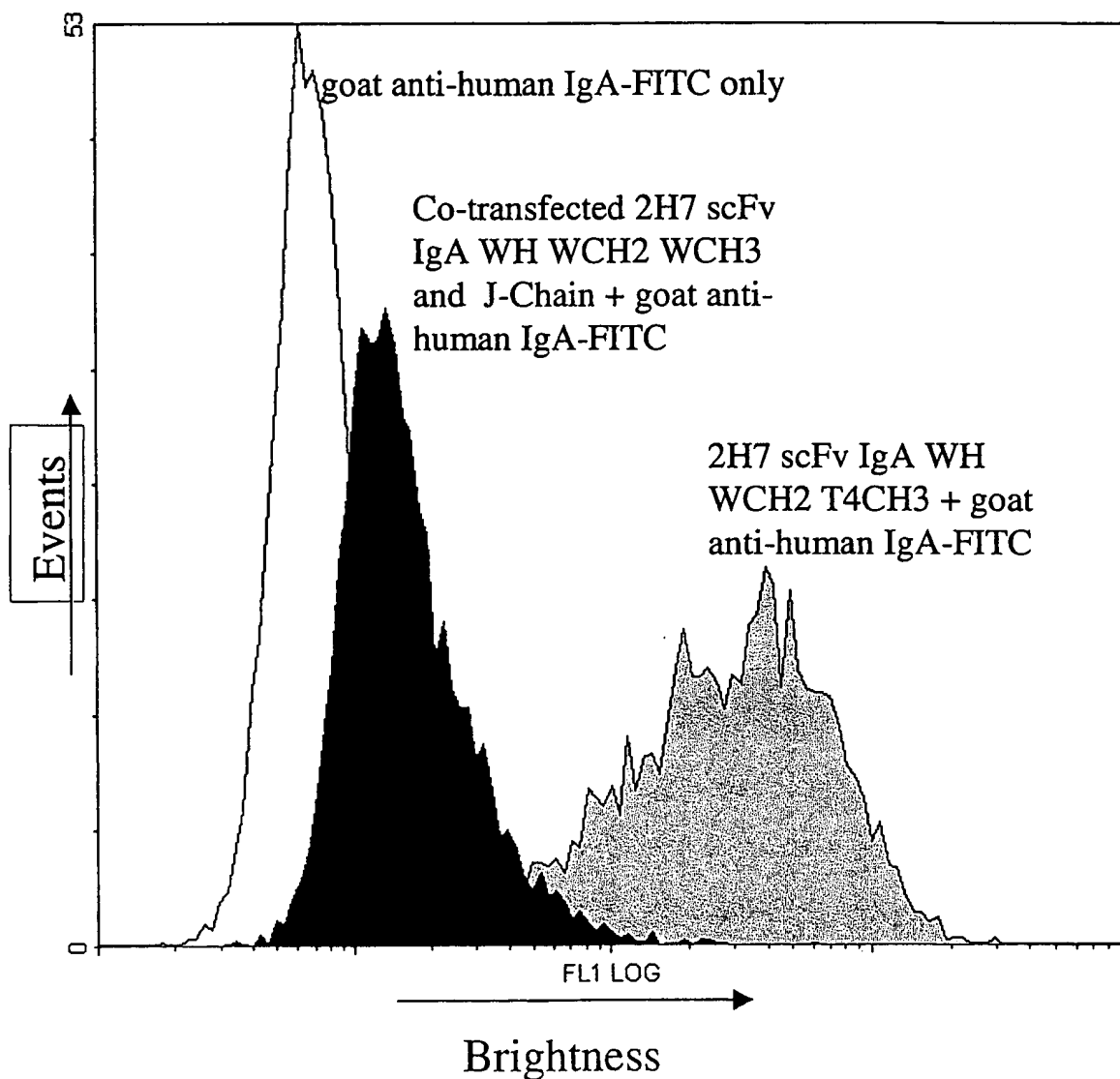


Fig. 37

Titration of CD20 specific scFvIg Constructs  
for ADCC Activity Using Whole Blood Effectors

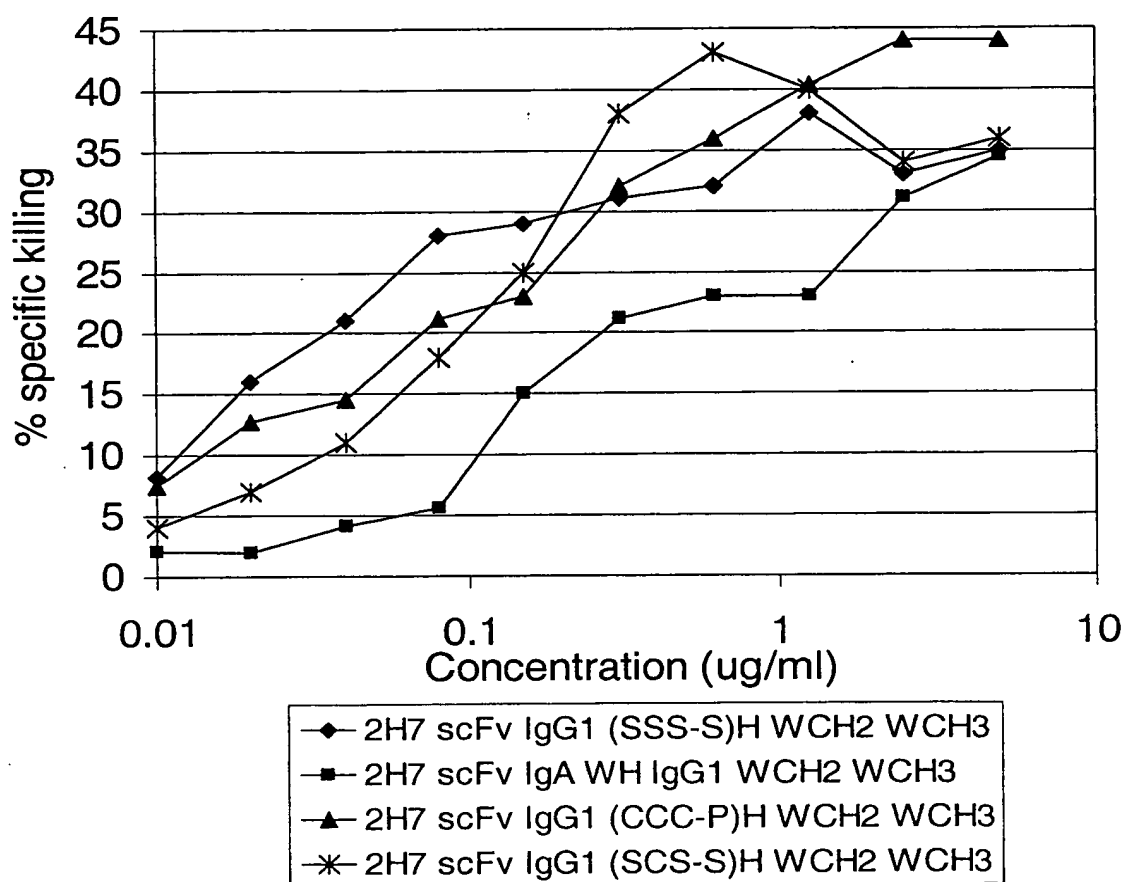
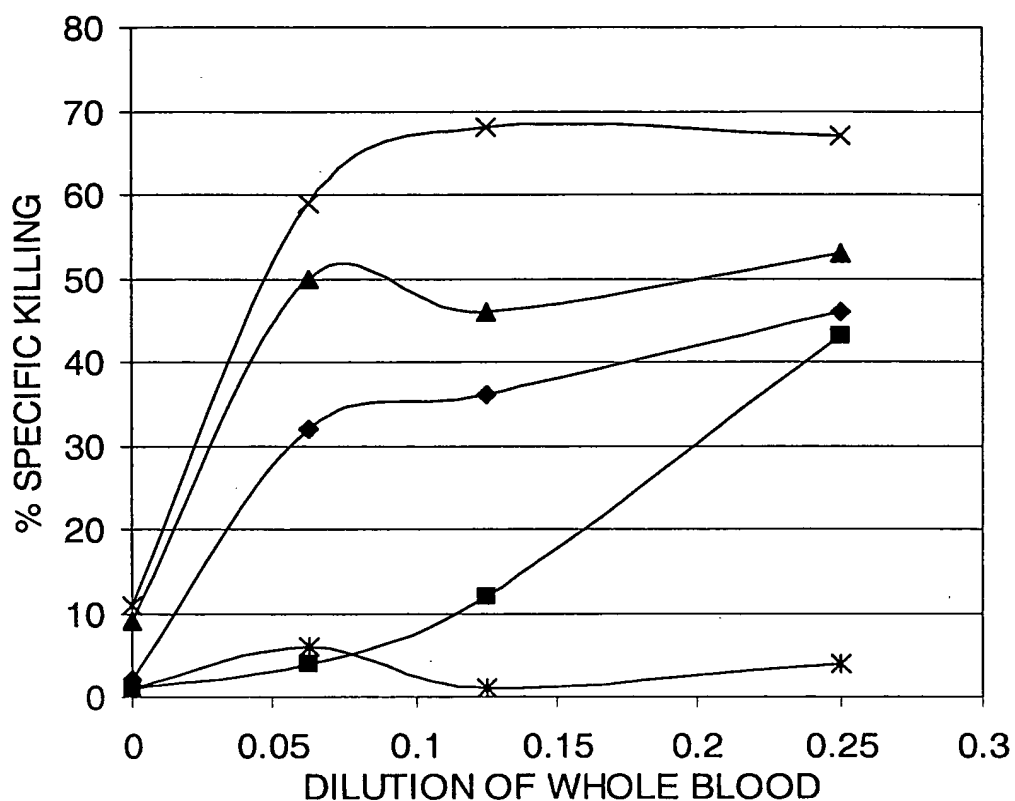


Fig. 38

ADCC Assay of anti-CD20 constructs with alternative tails  
(Whole Blood Effectors / BJAB Targets)



- ◆ 2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3
- 2H7 scFv IgA WH WCH2 T4CH3
- ▲ 2H7 scFv hlgG1 (SCS-S)H WCH2 WCH3
- ✕ 2H7 scFv hlgG1 (CCC-P)H WCH2 WCH3
- \* Effectors Only

Fig. 39

ADCC Assay of Anti-CD20 scFvIg Constructs  
Using Different Effector Populations Against BJAB Targets

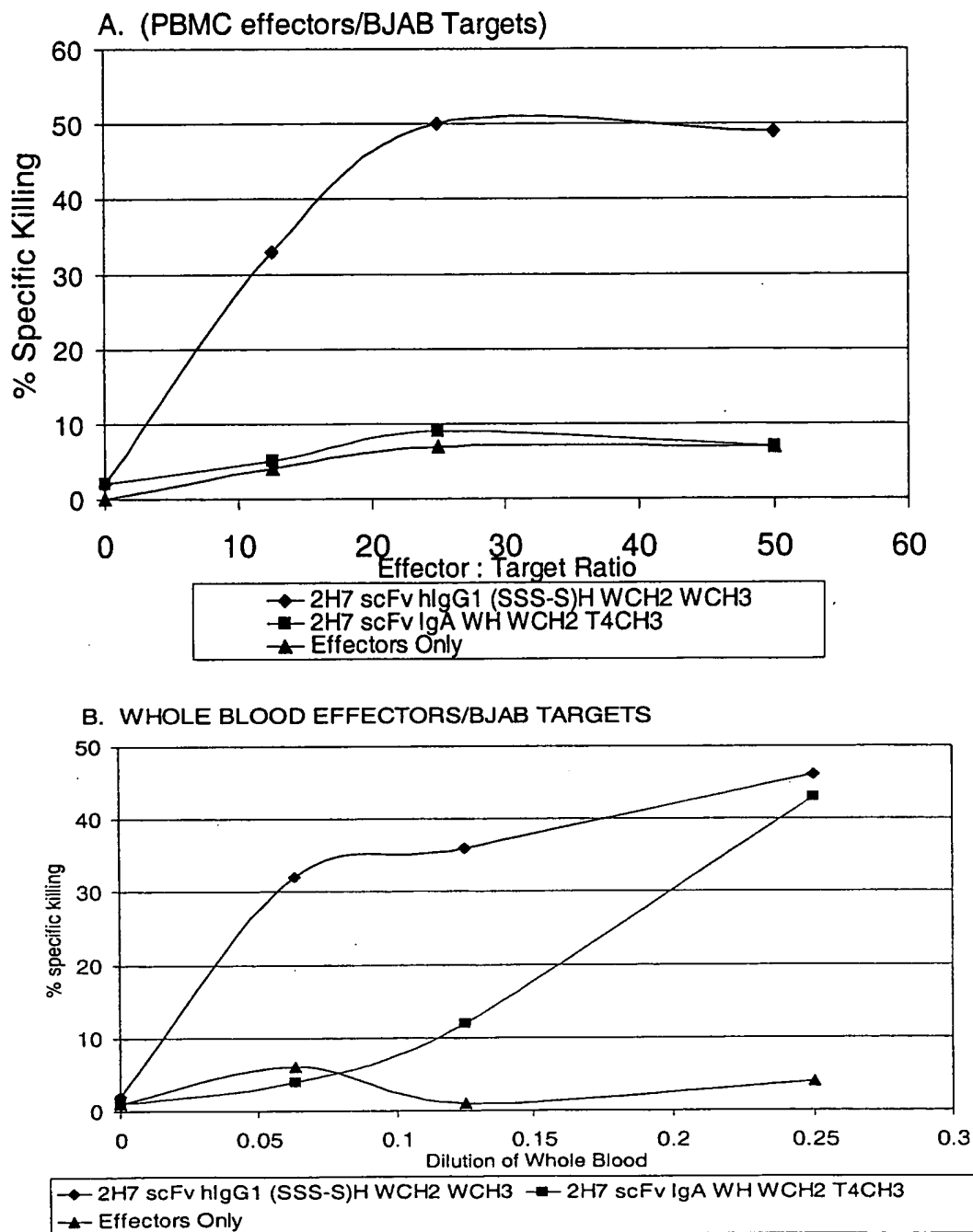
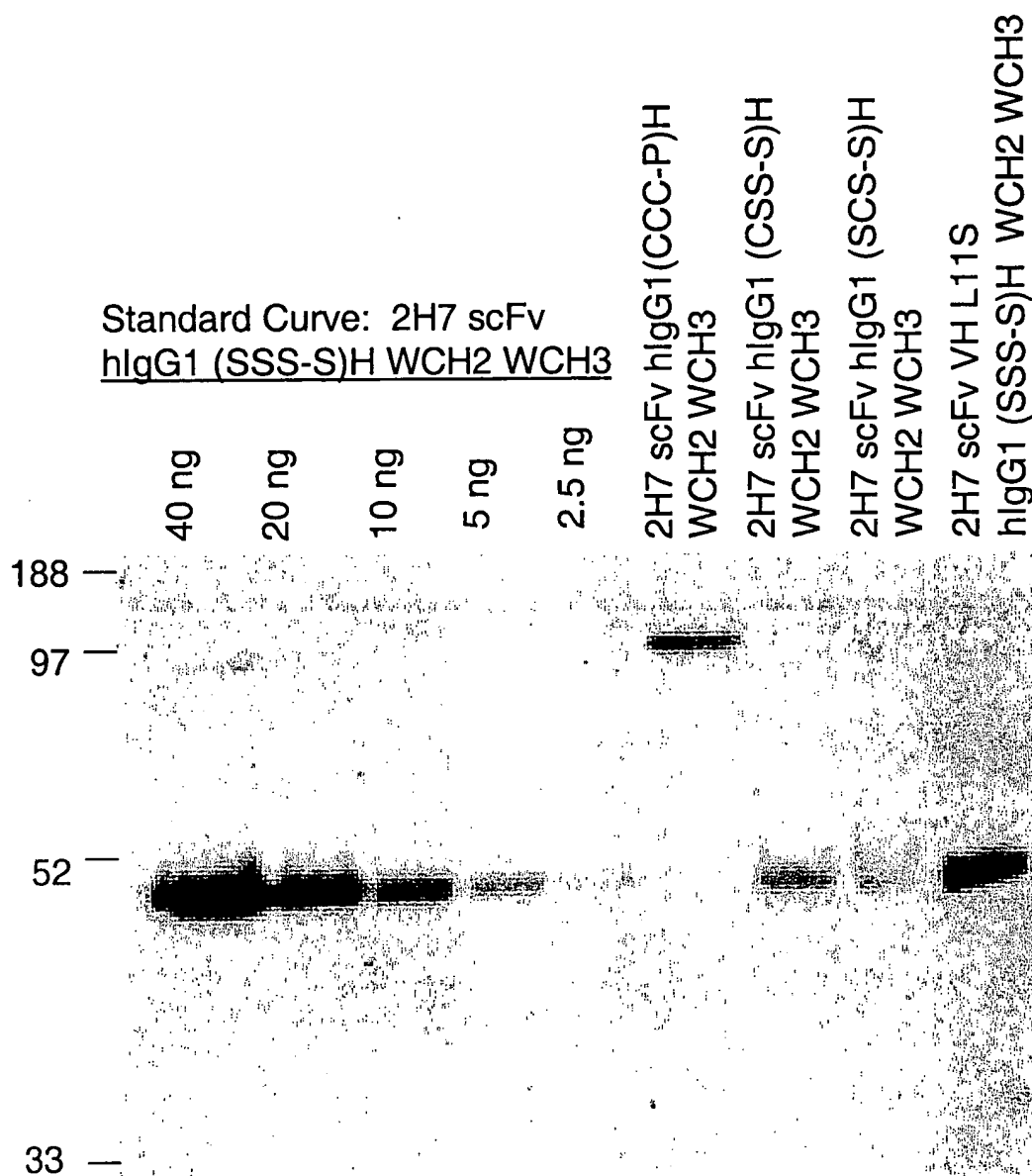


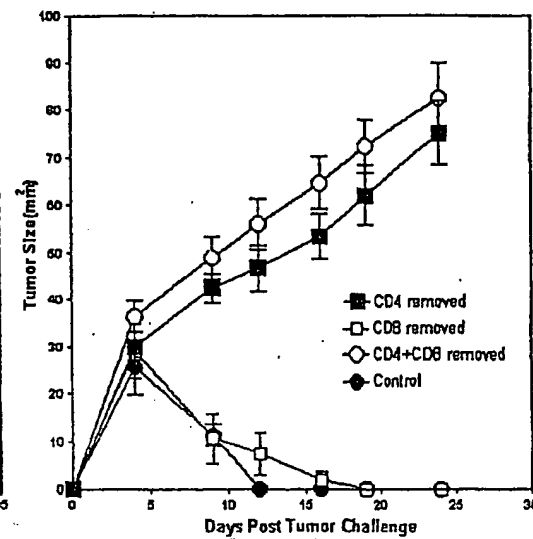
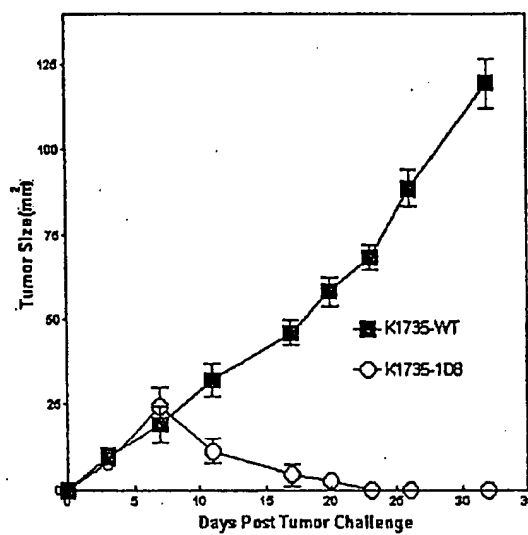
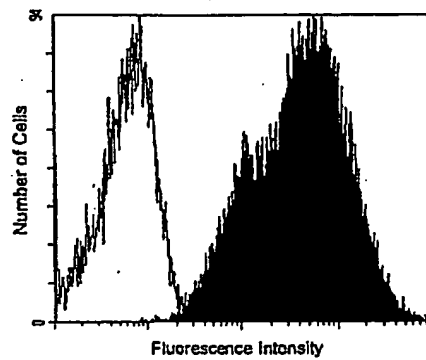
Fig. 40

Immunoblot of 2H7 scFv Ig constructs from COS  
Transfections (1  $\mu$ l/well) compared to a Concentration Standard



Figures 41A, 41B and 41C

A.



Sheet 45 of 53

B.

C.

Fig. 42

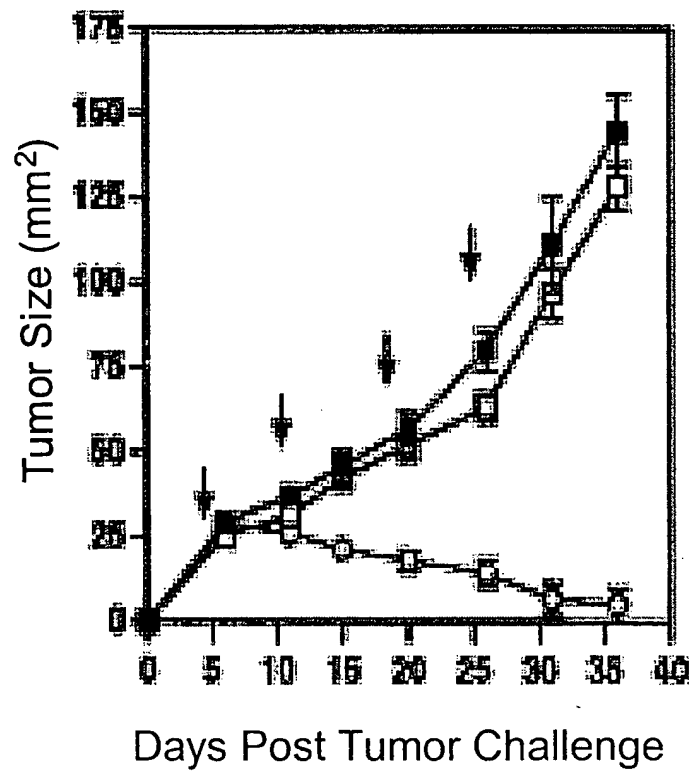




Fig. 43

Mixtures of K1735-WT and K1735-1D8 transfected tumor lines  
inhibit tumor outgrowth in C3H mice

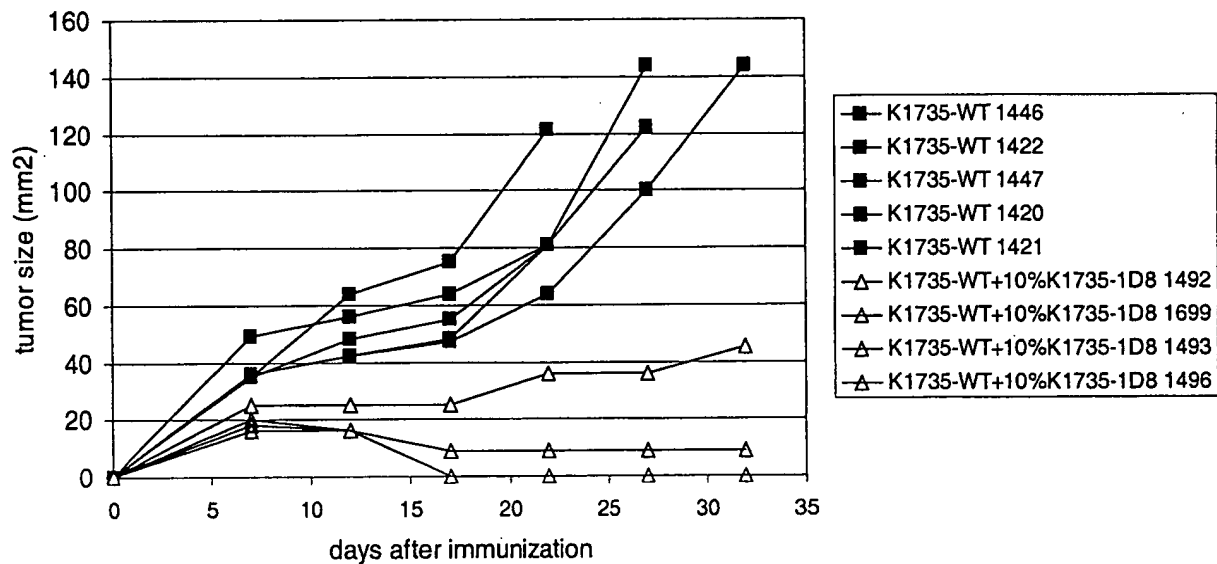


Fig. 44

Expression of anti-mouse CD137 (1D8) scFv-hIgG1 (SSS-S)H P238SCH2 WCH3  
On the surface of panned Ag104-1D8 Transfected Tumor Cells

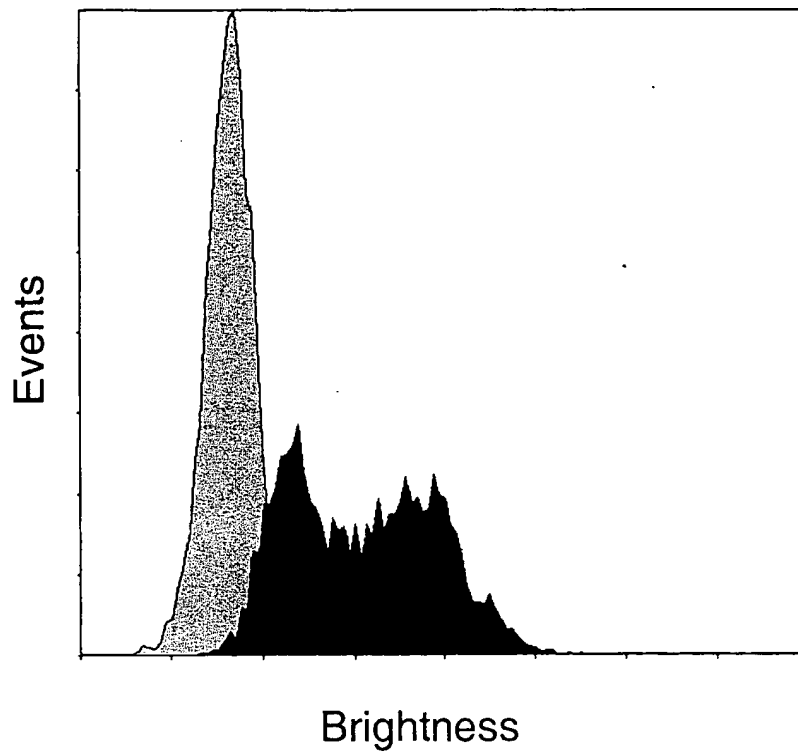


Fig. 45  
Coomassie Stained SDS-PAGE Gel of 2H7 scFv Ig  
Constructs

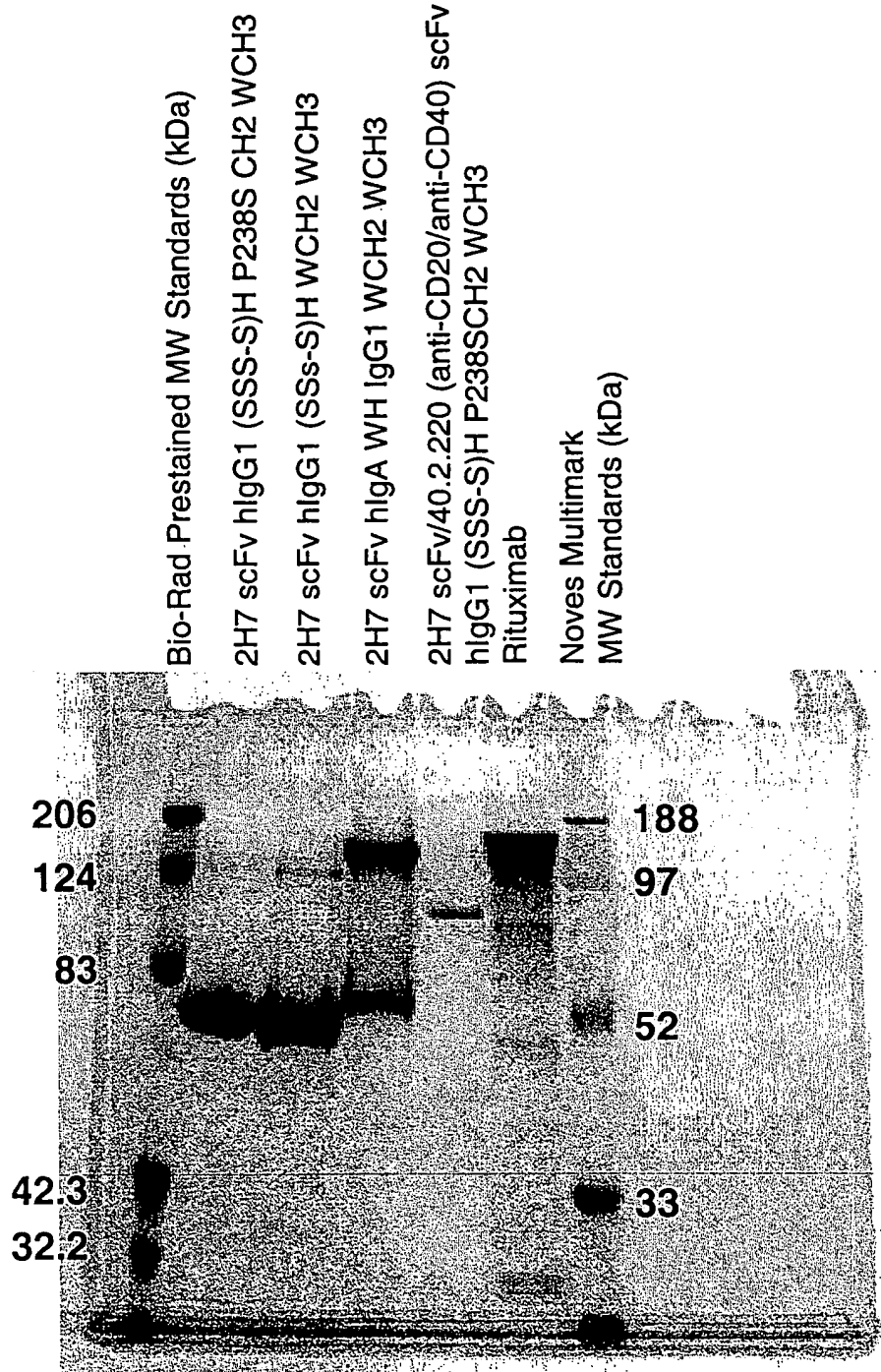
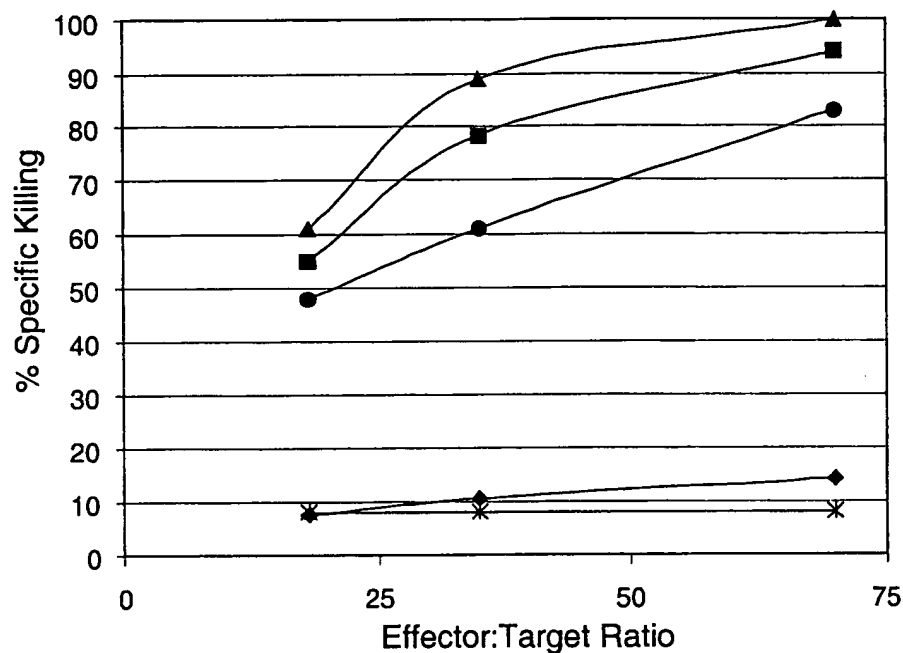


Fig. 46  
ADCC mediated by 2H7 scFvIg Constructs by human  
PBMC effector cells against Bjab targets

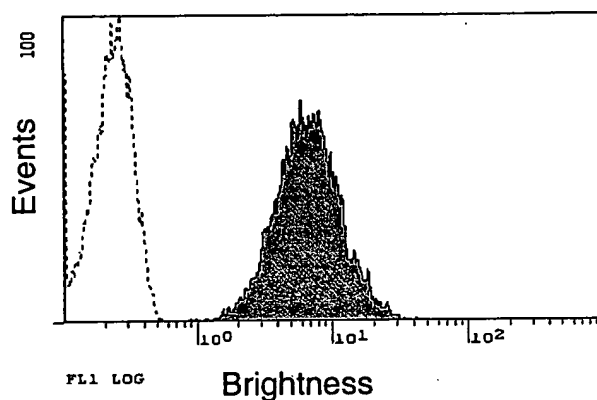


- |  |                        |
|--|------------------------|
| ◆ 2H7 scFv hlgG1(SSS-S)H P238SCH2 WCH3 | ● RITUXIMAB            |
| ▲ 2H7 scFv hlgA WH IgG1 WCH2 WCH3      | * CELLS ALONE (W/O AB) |
| ■ 2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3    |                        |

Fig. 47

Cell surface expression of anti-human CD3 G19-4  
scFv hIgG1 (SSS-S)H P238SCH2 WCH3-  
hCD80TM/CT on Reh and T51 Cells.

Reh anti-CD3 (G19-4) scFv hIgG1 (SSS-S)H  
P238SCH2 WCH3-hCD80TM/CT



T51 G19-4 scFv hIgG1 (SSS-S)H  
P238SCH2 WCH3-hCD80TM/CT:

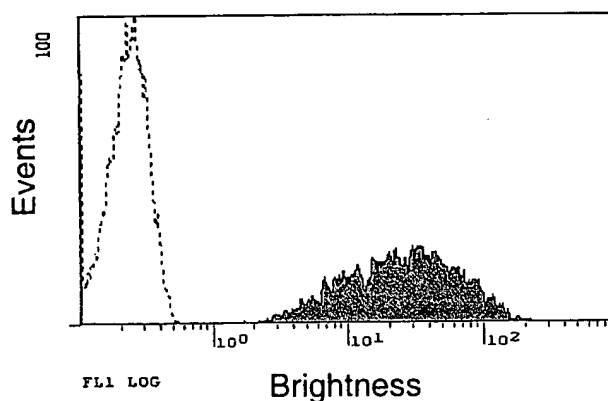
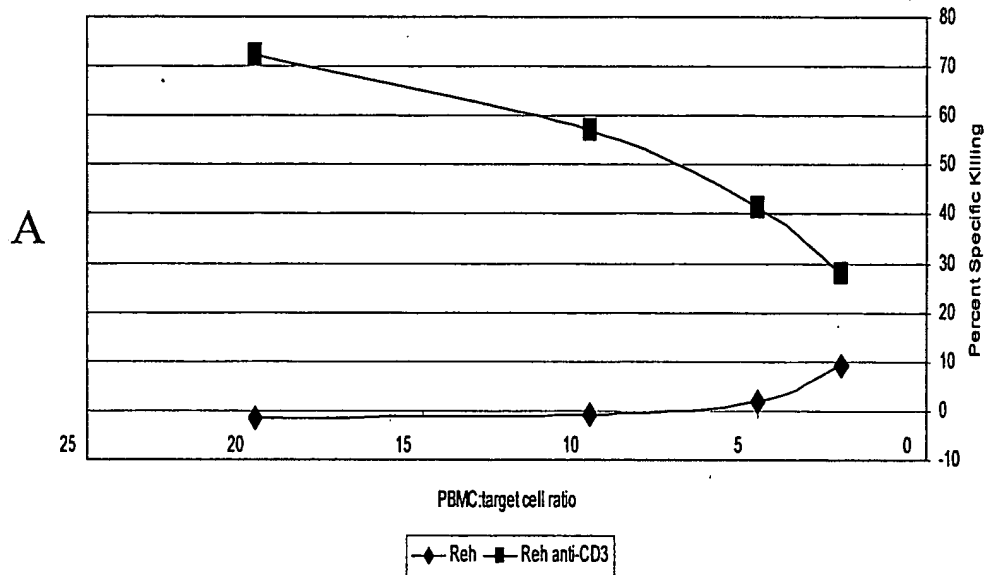


Figure 48.  
Targeting of Cytotoxicity to Transfected Cell Lines  
by Surface expression of CD3 scFvIg  
Cytotoxic activity of resting PBMC towards transfected Reh cells



Cytotoxic activity of resting PBMC towards transfected T51 lymphoblastoid cells

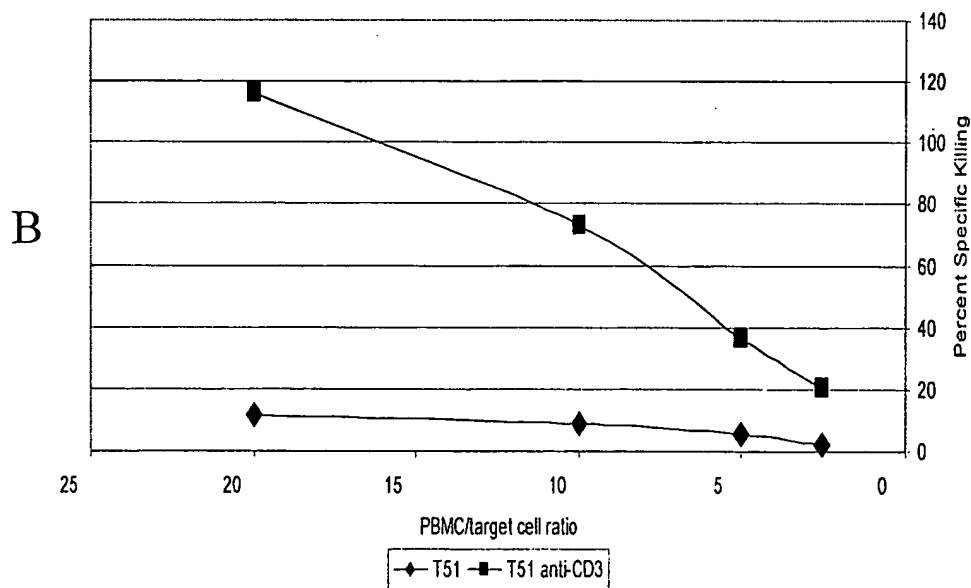


Fig. 49  
Binding of 5B9, a mouse anti-human CD137 scFv hIgG1  
(SSS-S)H WCH2WCH3 to stimulated human PBMC

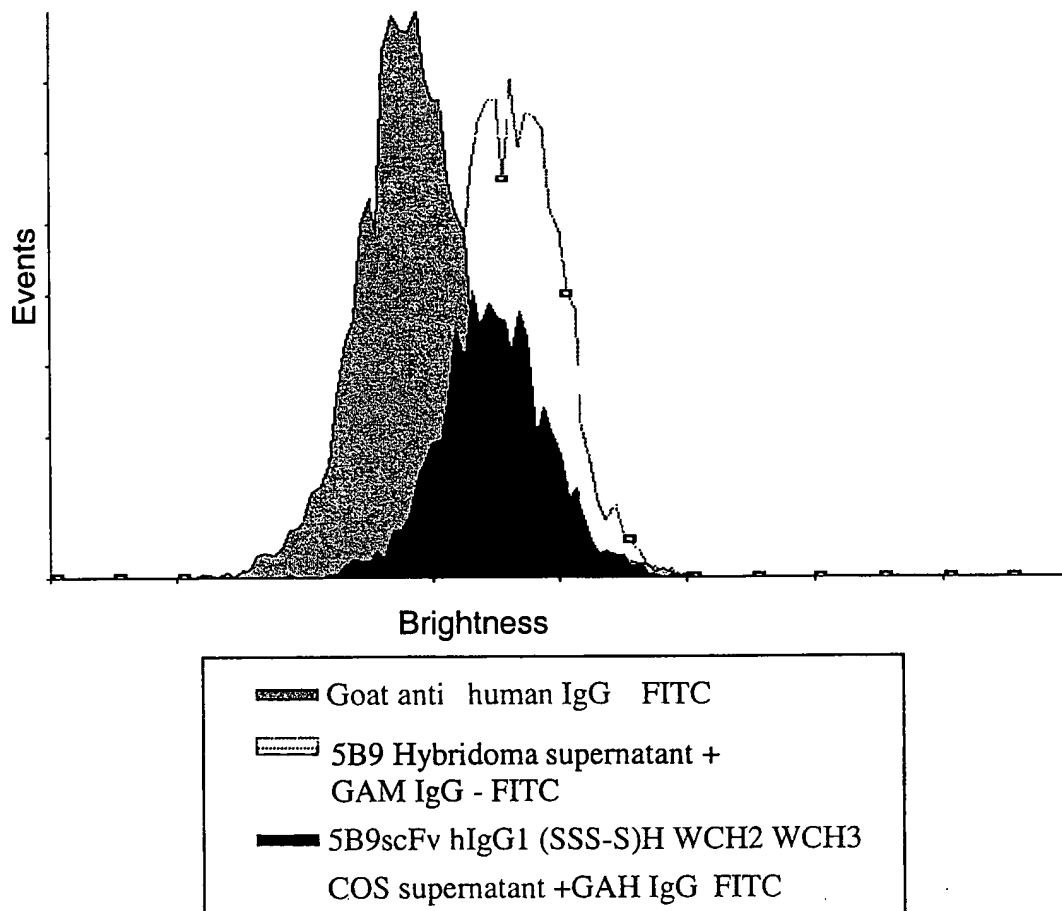
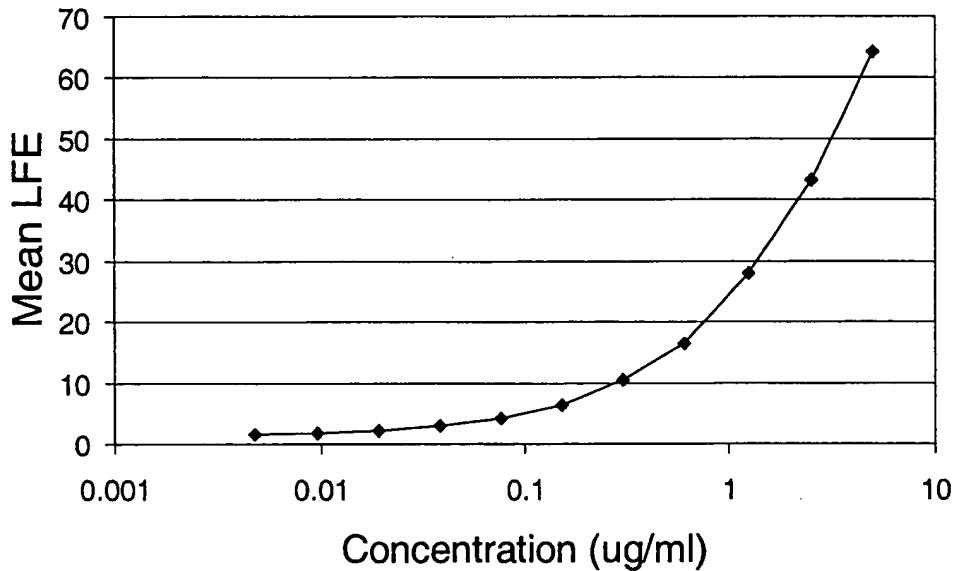


Fig. 50

Effect of V<sub>H</sub>L11S Mutation on CytosB20  
2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3 Protein Expression

50A. Standard Curve: 2H7VH-L11S-IgG1 (SSS-S)H WCH2 WCH3



50B. CHO supernatant Brightness and Estimation of Protein concentrations from Standard Curve:

	CHO clone name				
	4F2	4F5	3E5	6B11A	2B8A
Mean LFE					
1/100	71.7	40.6	31.5	99.7	101.5
1/500	27.1	12.4	11.2	40.8	43
approx conc. μg/ml	600	225	125	1000	1250



Fig. 51  
Production Levels of 2H7scFv VH L11S hIgG1  
(SSS-S)H WCH2 WCH3  
From CHO Clone Culture Supernatants

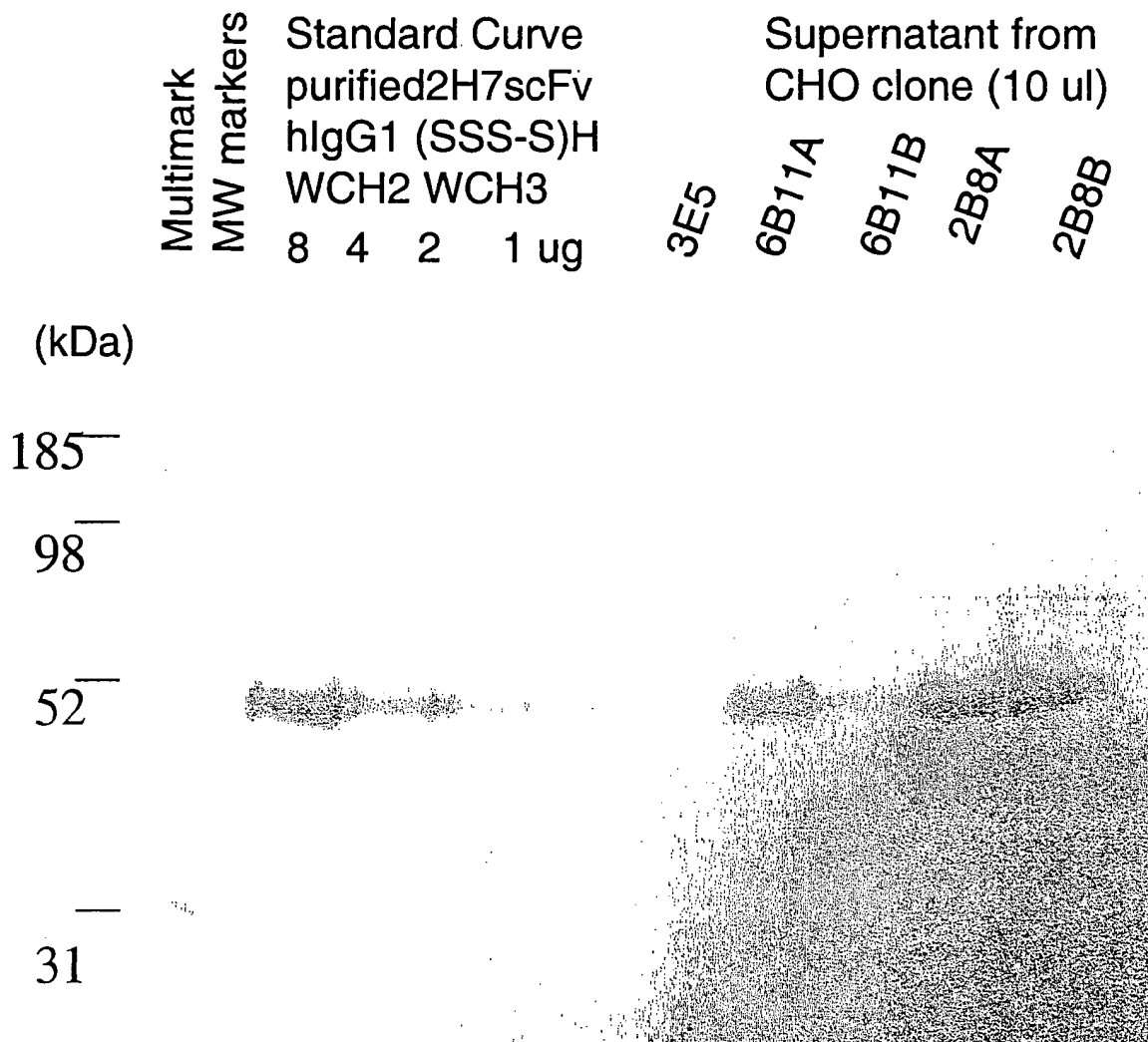
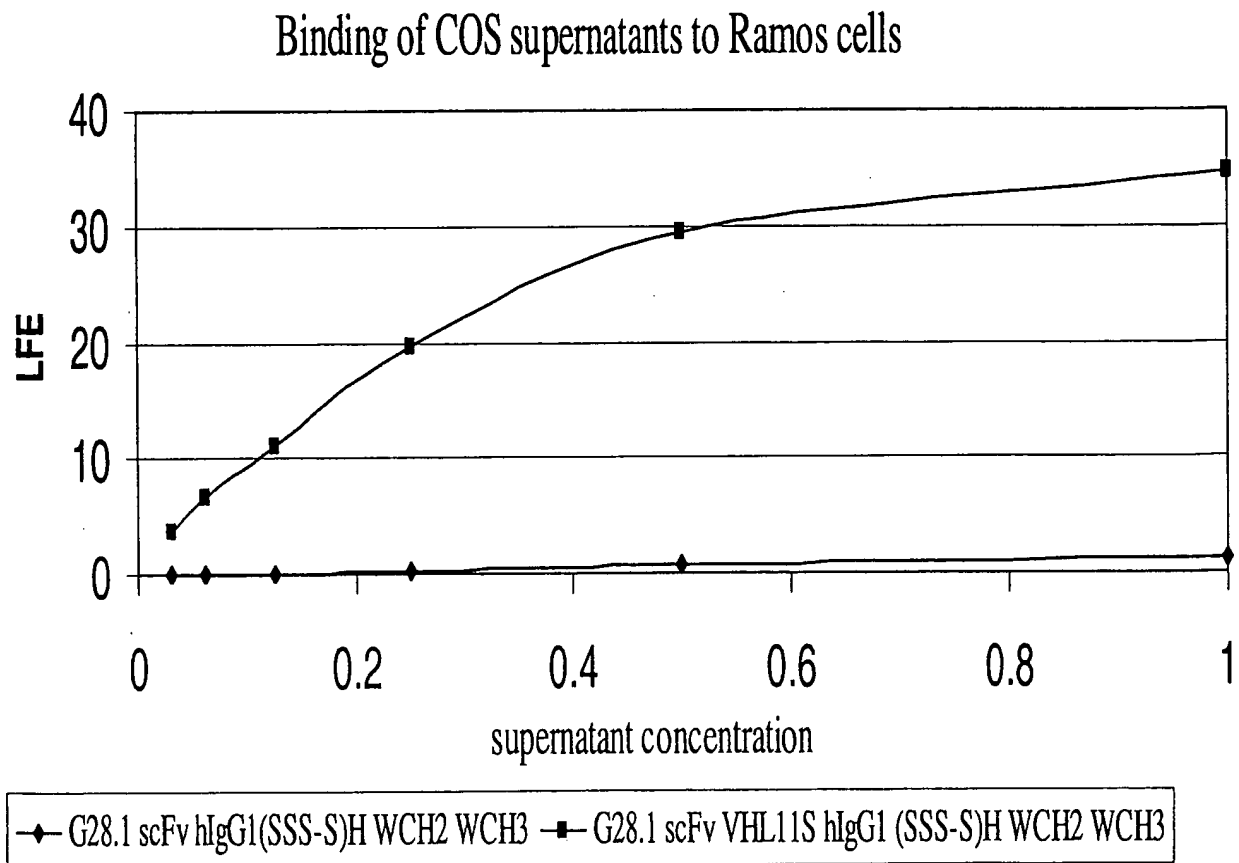


Fig. 52  
Effect of VHL11S Mutation on G28-1 scFvIg Construct  
Protein Production from COS cells



## Fig. 53

### Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants  
transfected with G28-1 scFv hlgG1 (SSS-S)H WCH2 WCH3  
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Fig. 53A.

Purified G28-1 (11/6/01)	G28-1 scFv hlgG1 (SSS-S)H				
scFv IgG1 (SSS-S)H	WCH2 WCH3				
WCH2 WCH3	1 ul/well				
80ng	40ng	20ng	10ng	A	B C D E

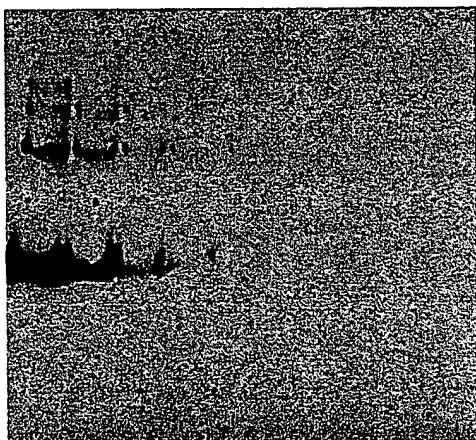


Fig. 53B.

Purified G28-1 (11/6/01)	G28-1VHL11S scFv hlgG1 (SSS-S)H				
scFv hlgG1(SSS-S)H	WCH2 WCH3				
WCH2 WCH3	1 ul/well				
80ng	40ng	20ng	10ng	A	B C D E

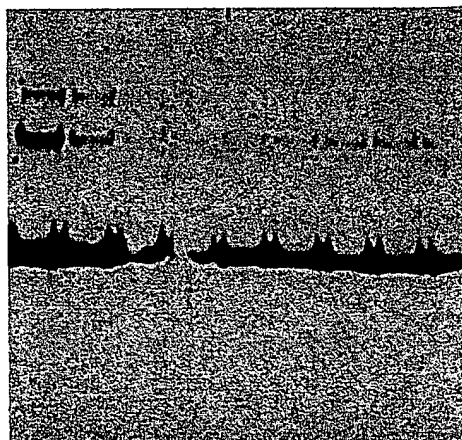


Fig. 54

Binding of 2H7 scFvIg Constructs with Altered  
Hinges and CH3 domains to CD20 CHO Cells

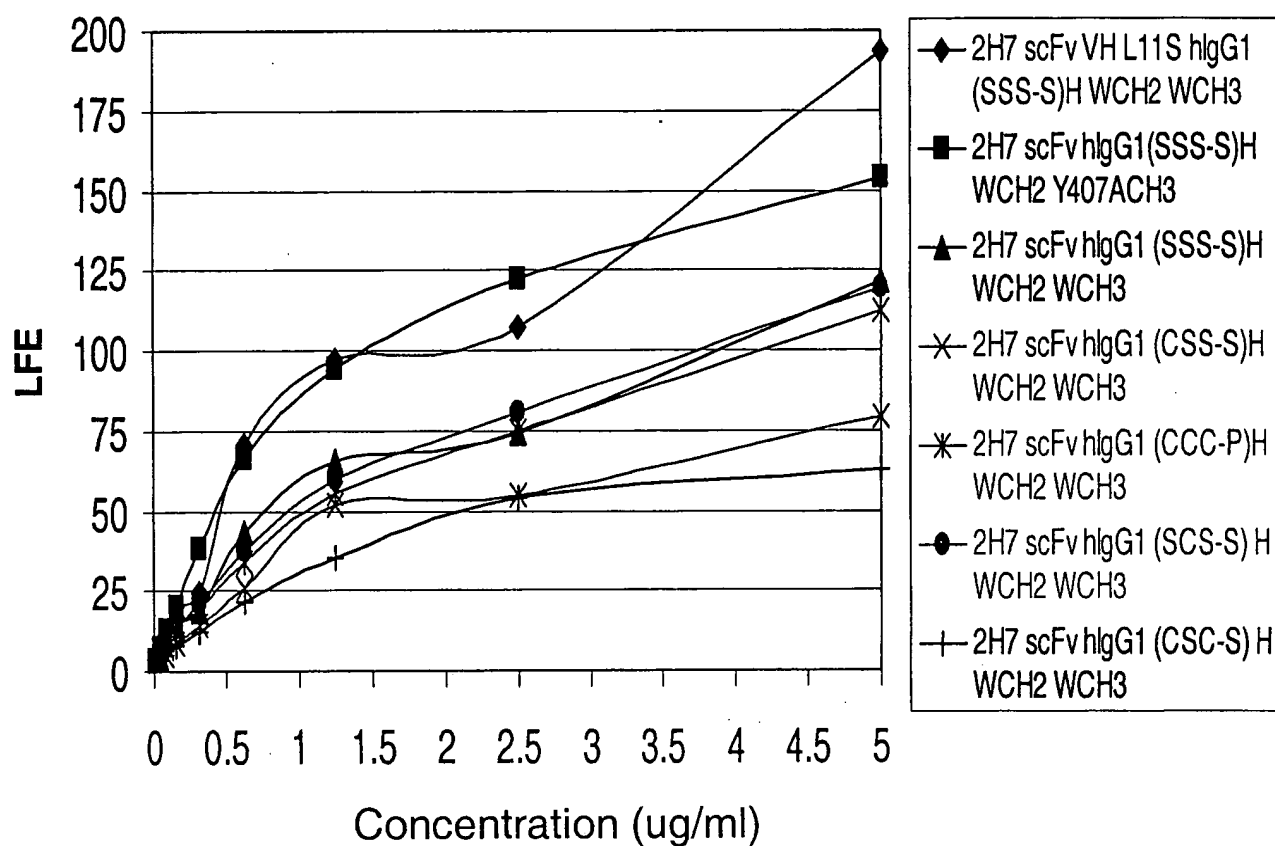


Fig. 55

ADCC Activity of 2H7 scFvlg constructs Against  
BJAB Targets and PBMC Effectors

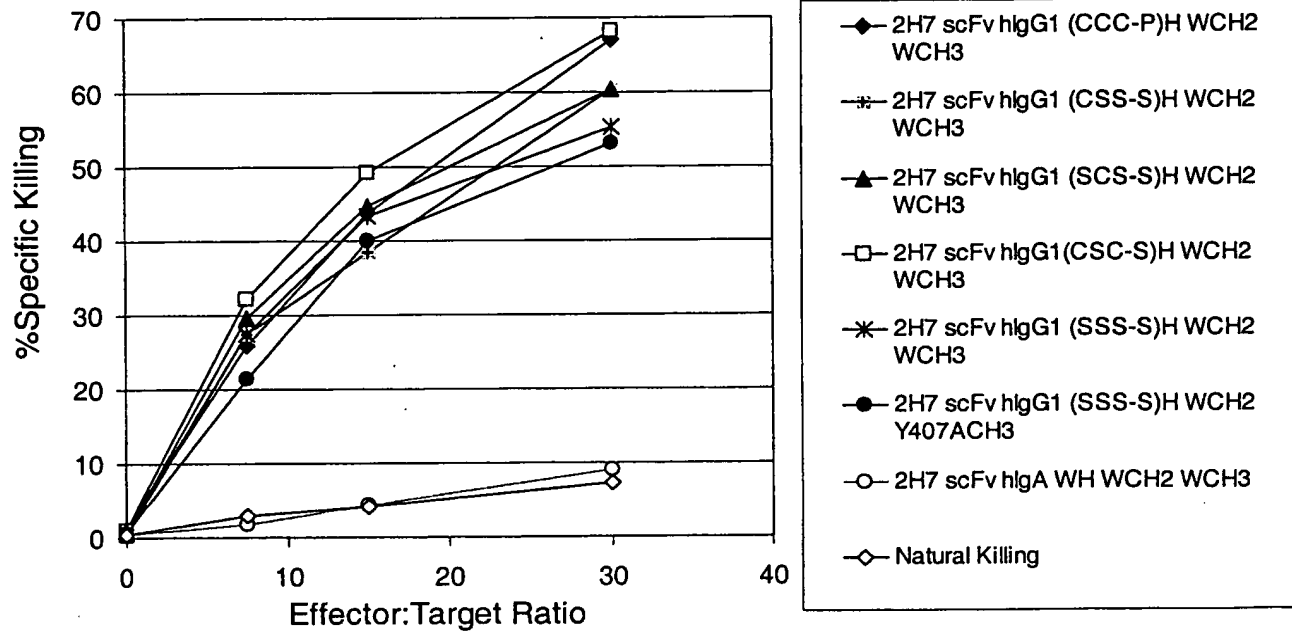


Fig. 56

Complement Activity of 2H7 scFvlg Constructs  
With Ramos Target Cells

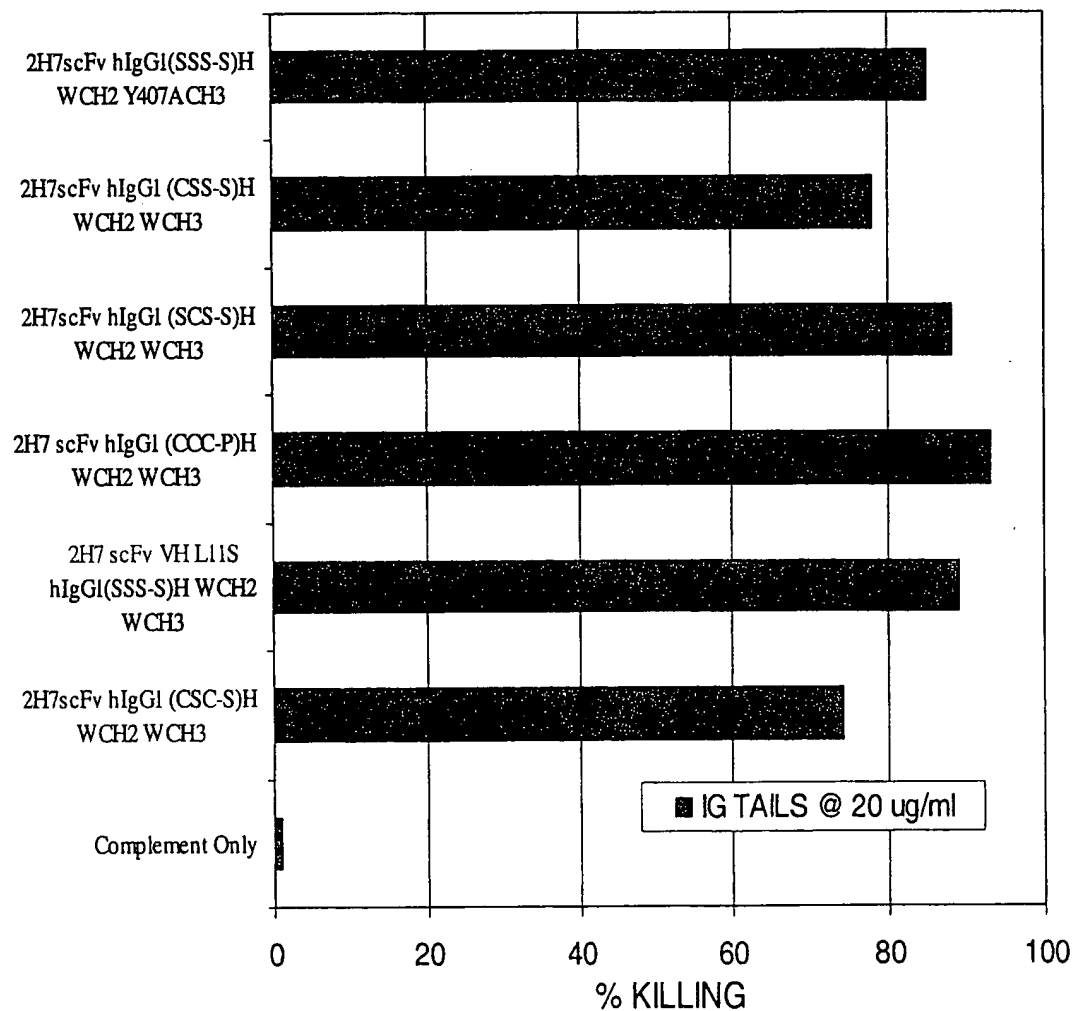
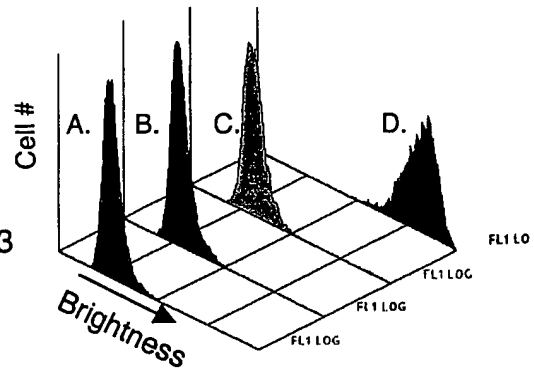


Fig. 57

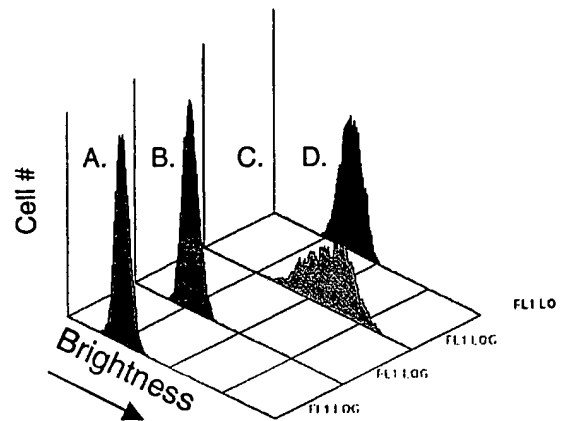
Binding of 2H7 scFvIg Derivatives to CD20CHO Cells

- A. ■ No fusion protein  
B. ■ 2H7 scFv hlgE CH2CH3CH4  
C. ■ 2H7 scFv hlgA WH WCH2 WCH3  
D. ■ 2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3

FITC anti-hlgG



FITC anti-hlgA



FITC anti-hlgE

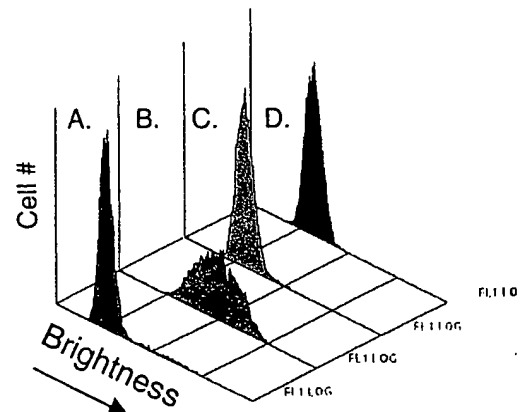


Fig. 58

Fig. 58A. 2H7 scFv VH L11S human IgE (WCH2 WCH3 WCH4)  
Binding to CD20 CHO at 30 ug/ml

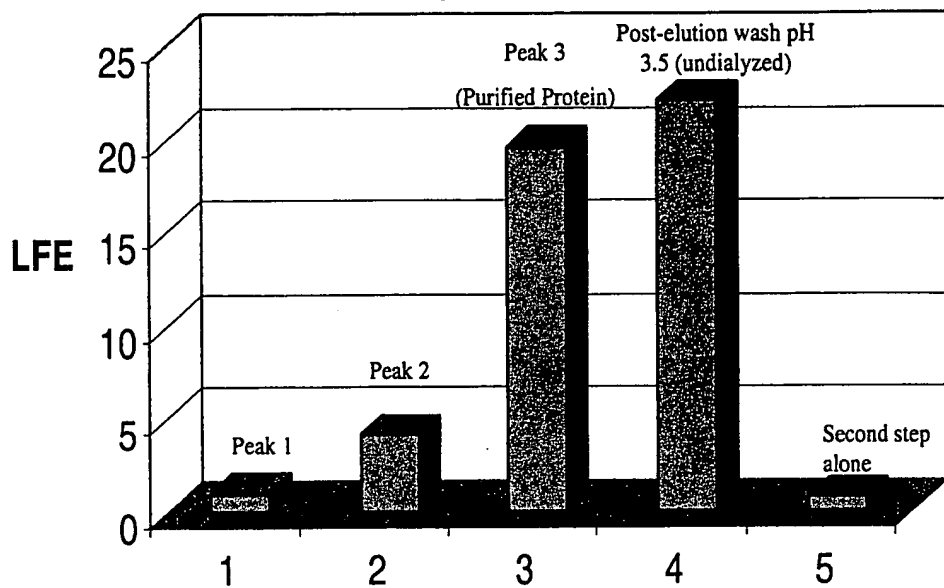


Fig. 58B. ADCC Activity of 2H7 VHL11S IgE (WCH2 WCH3 WCH4)  
Protein Fractions with **PBMC** Effectors and Bjab Targets

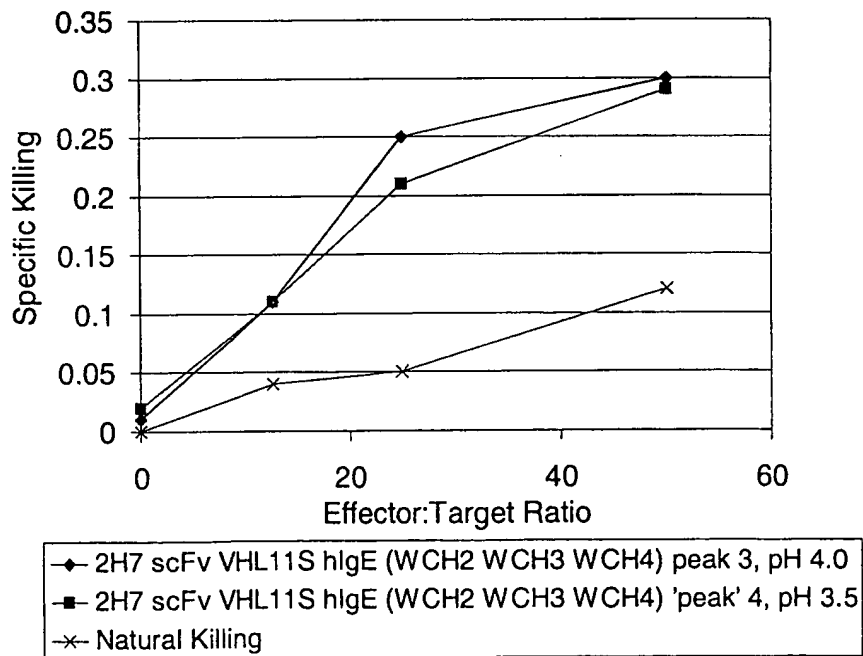




Fig. 59

Binding Data for COS derived  $\alpha$ -CD20 (2H7) scFv VHL11S  
mIg E (WCH2 WCH3 WCH4) and  
mIgA (WH WCH2 WCH3) Tailed Molecules

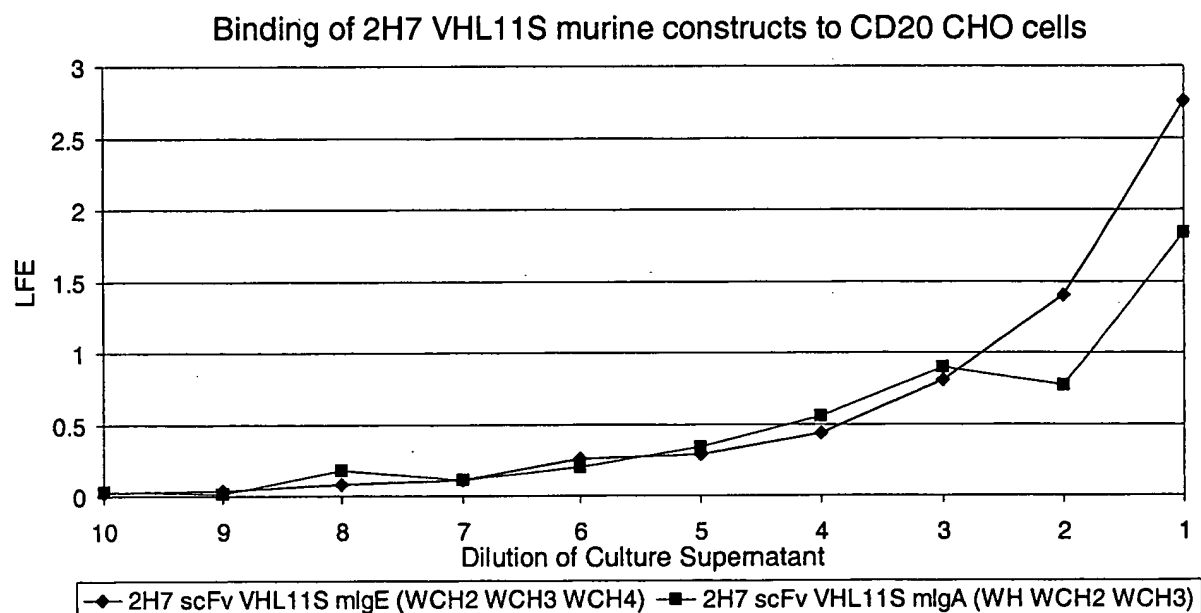
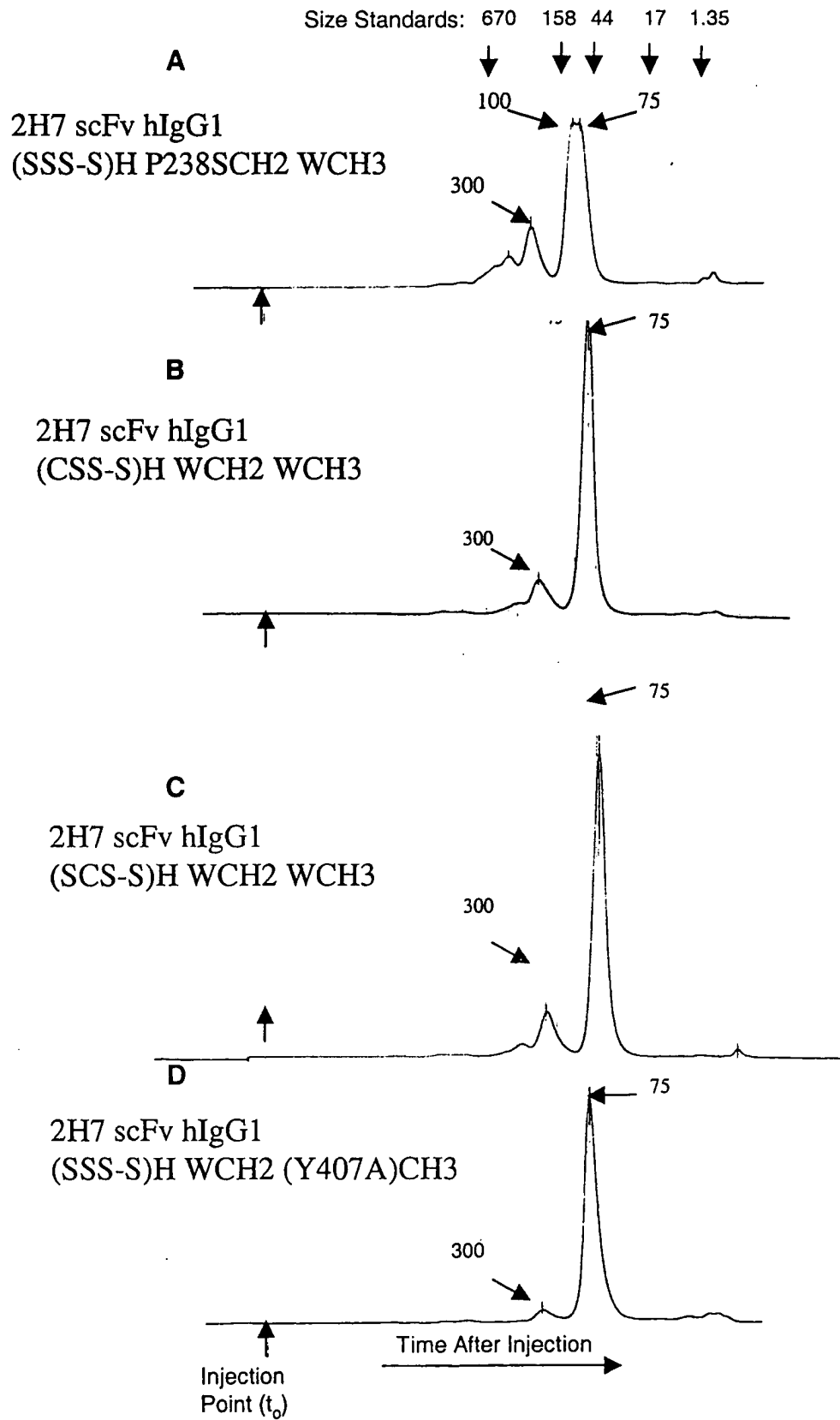


Fig. 60  
HPLC Profiles of 2H7 scFvIg Constructs



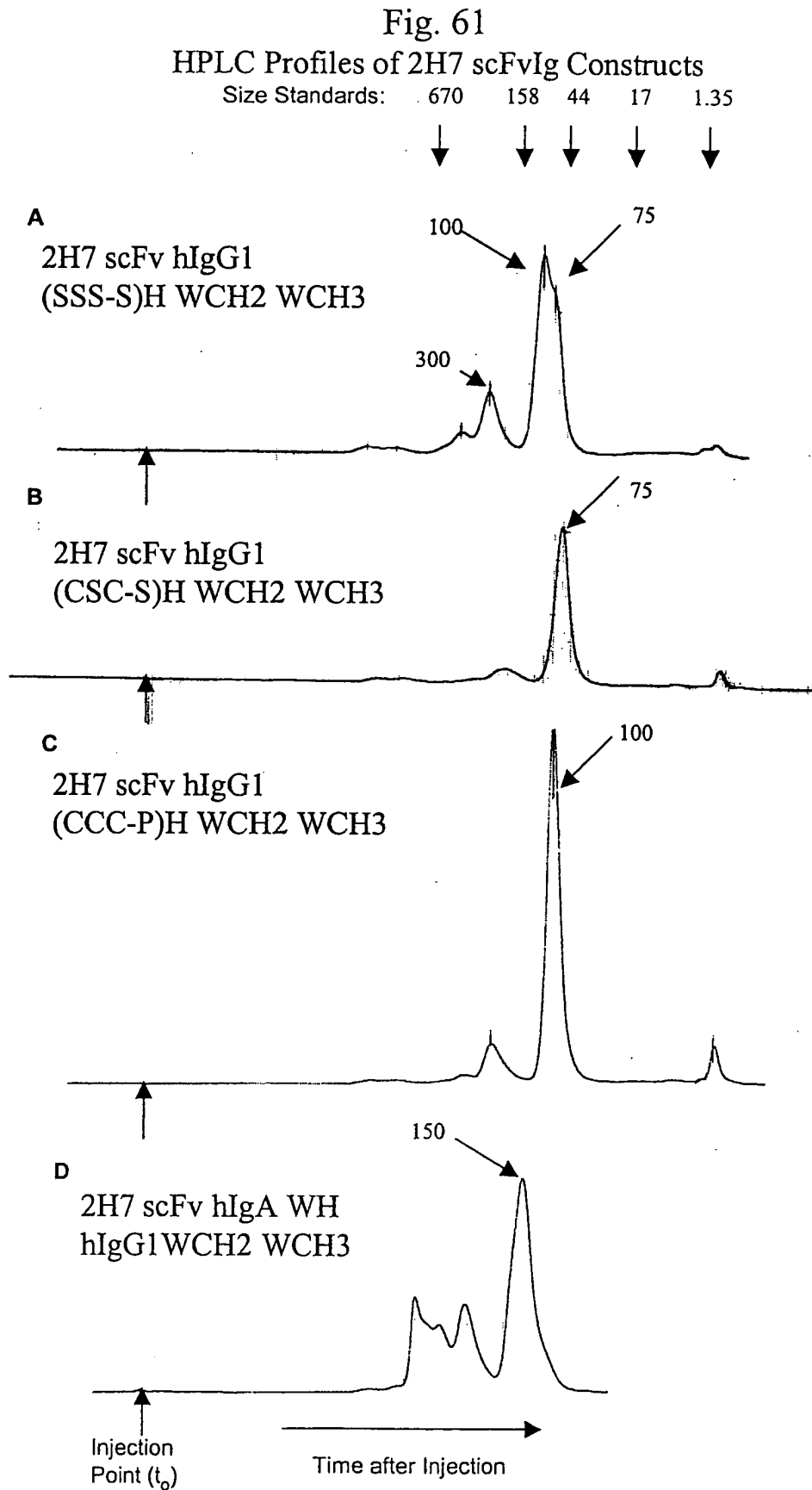


Fig. 62

HPLC Profiles of 2H7 scFvIg Constructs:  
Size Standards: 670 158 44 17 1.35

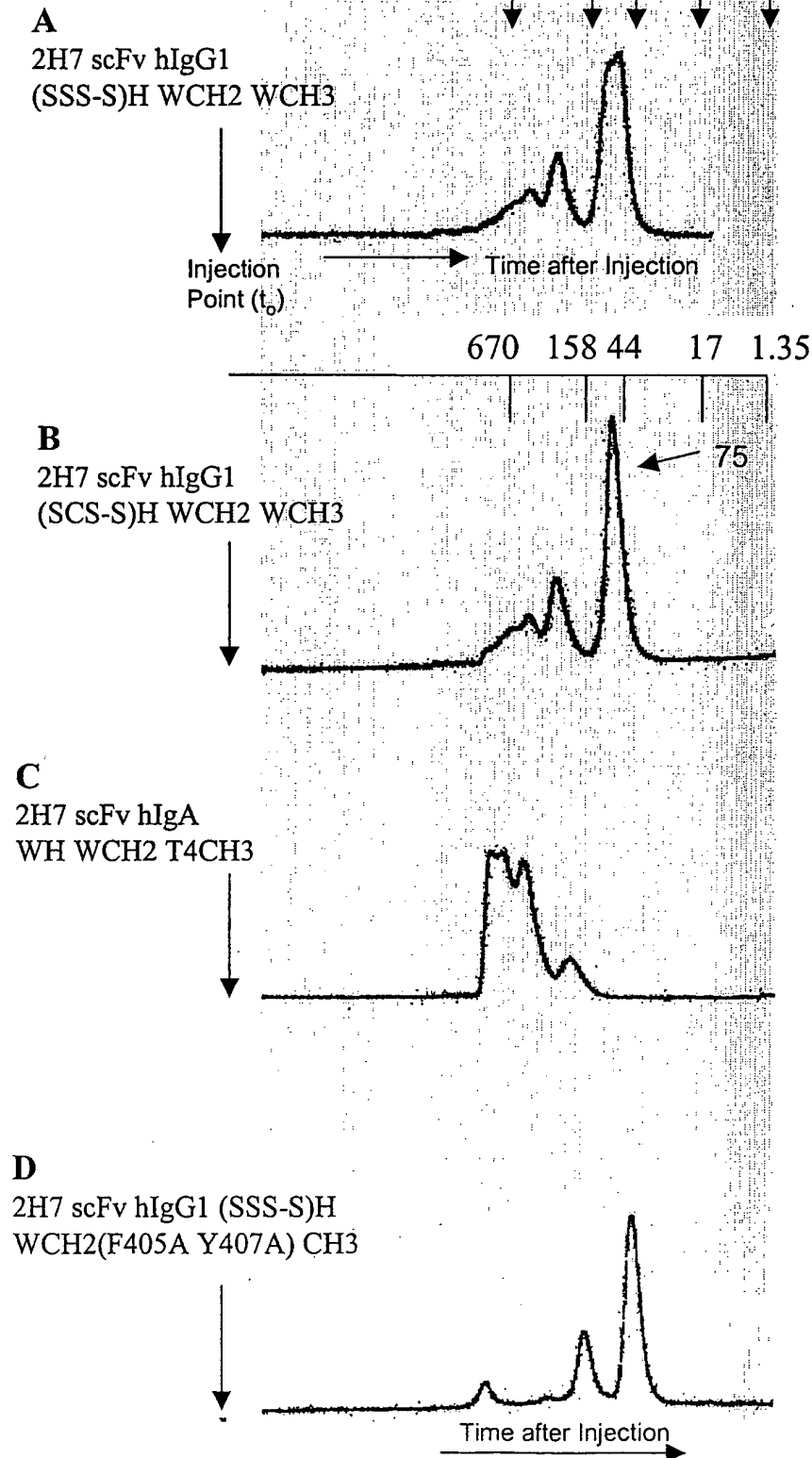


Fig. 63

Binding of Purified Proteins from COS Supernatants  
to CD20 CHO cells:  
Differential Effects of CH3 Mutations on Binding

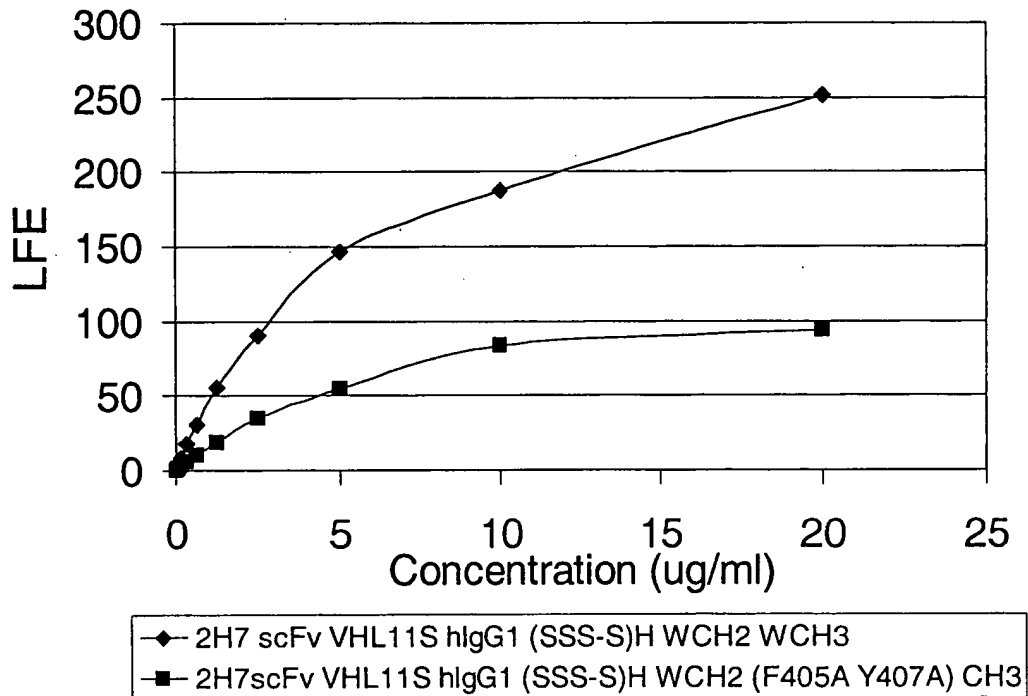


Fig. 64

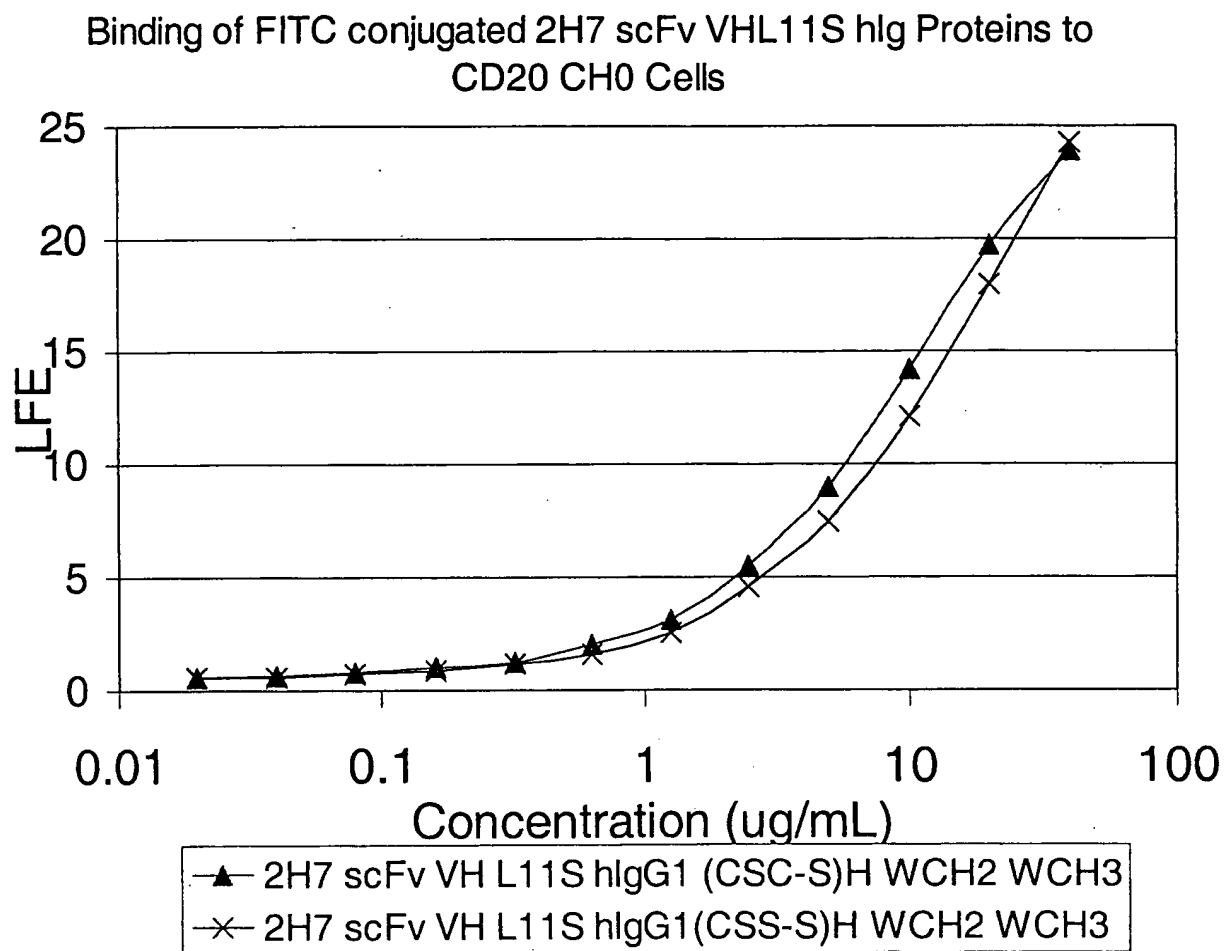


Fig. 65

Nonreducing SDS-PAGE on Protein A-Purified Lots  
of 2H7 scFv VHL11S hlg Constructs (10 ug/lane)

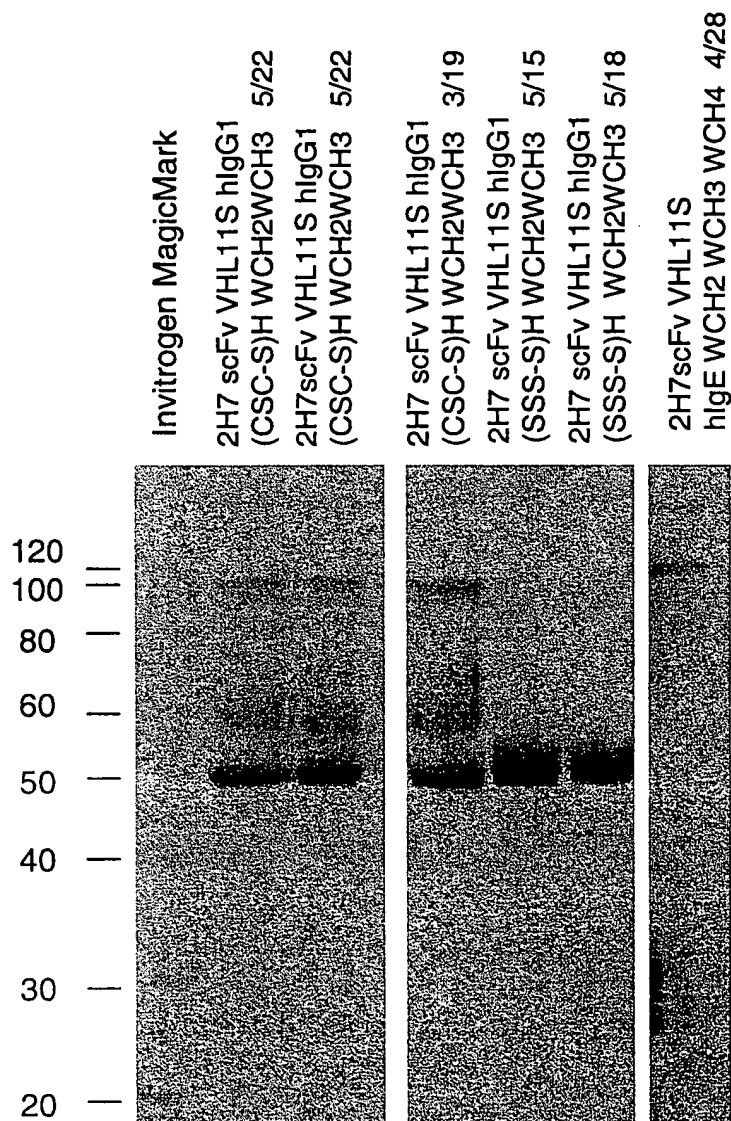


Fig. 66  
Alterations in Human IgG Fc sequence  
that differentially change effector function efficiency

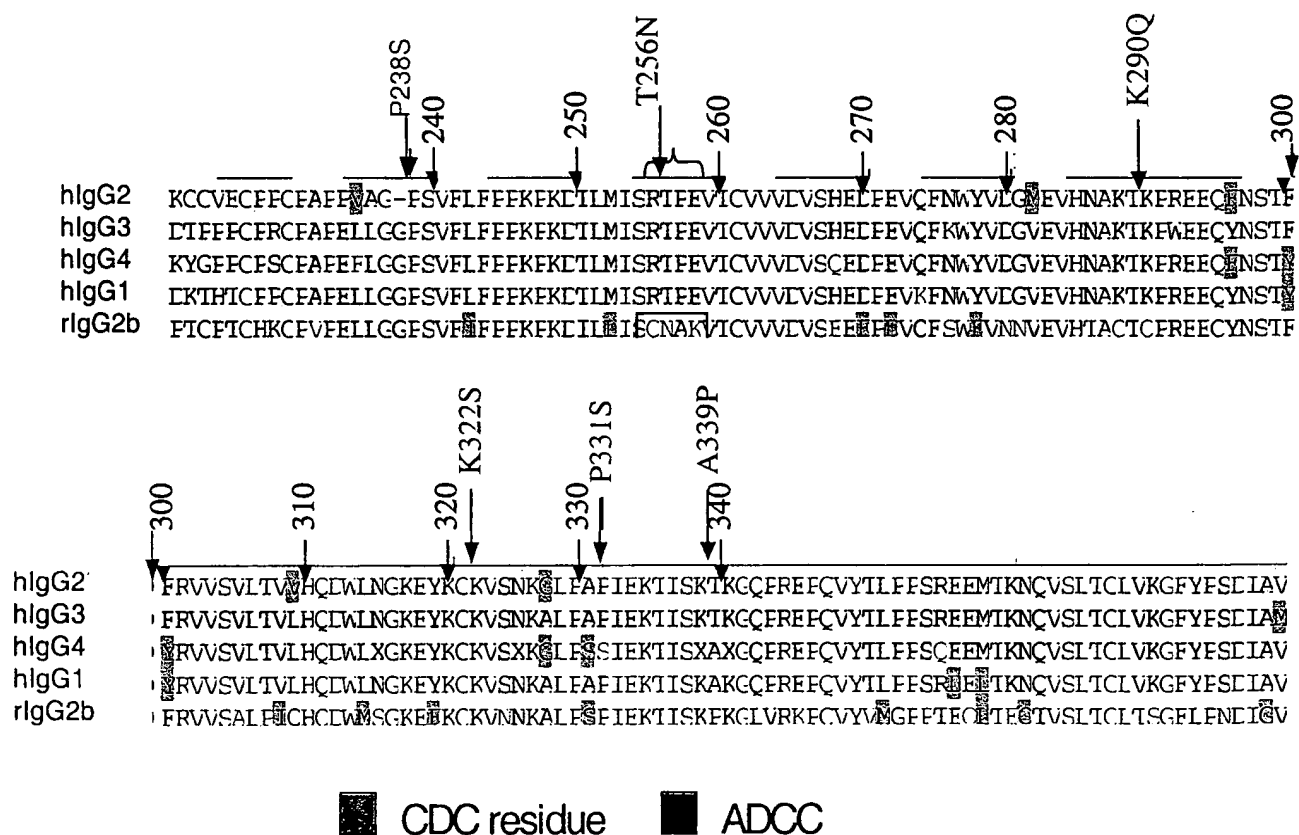




Figure 67.

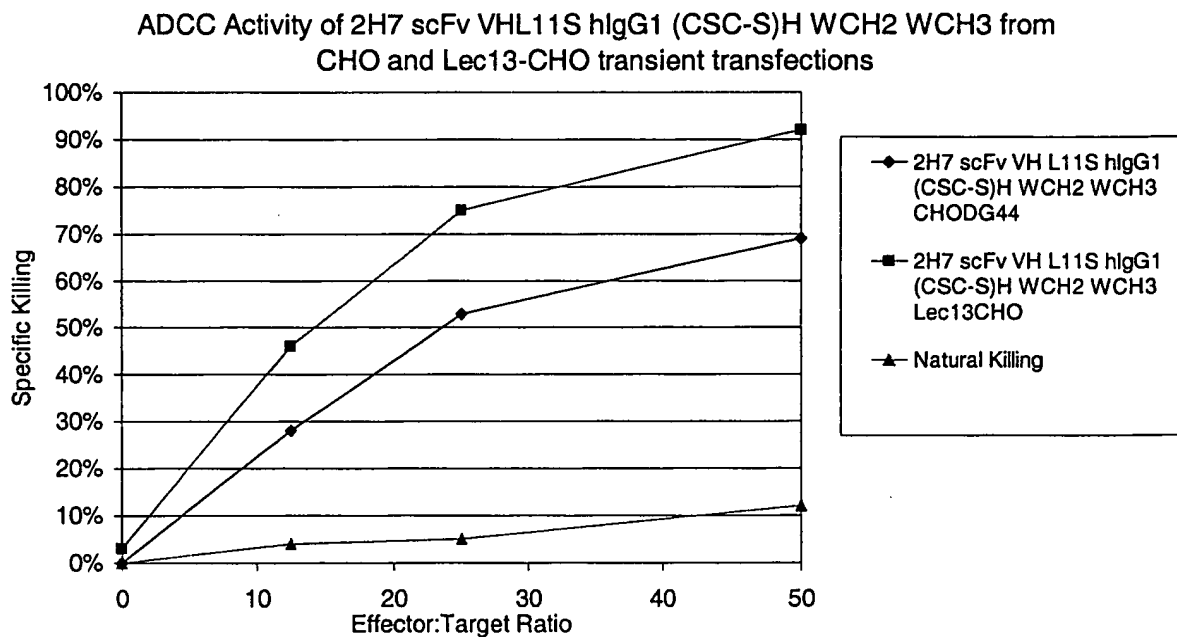


Fig. 68  
CD16(ED) hIgG1(SSS-S)H P238S CH2 WCH3 high and  
low affinity alleles expressed as soluble molecules

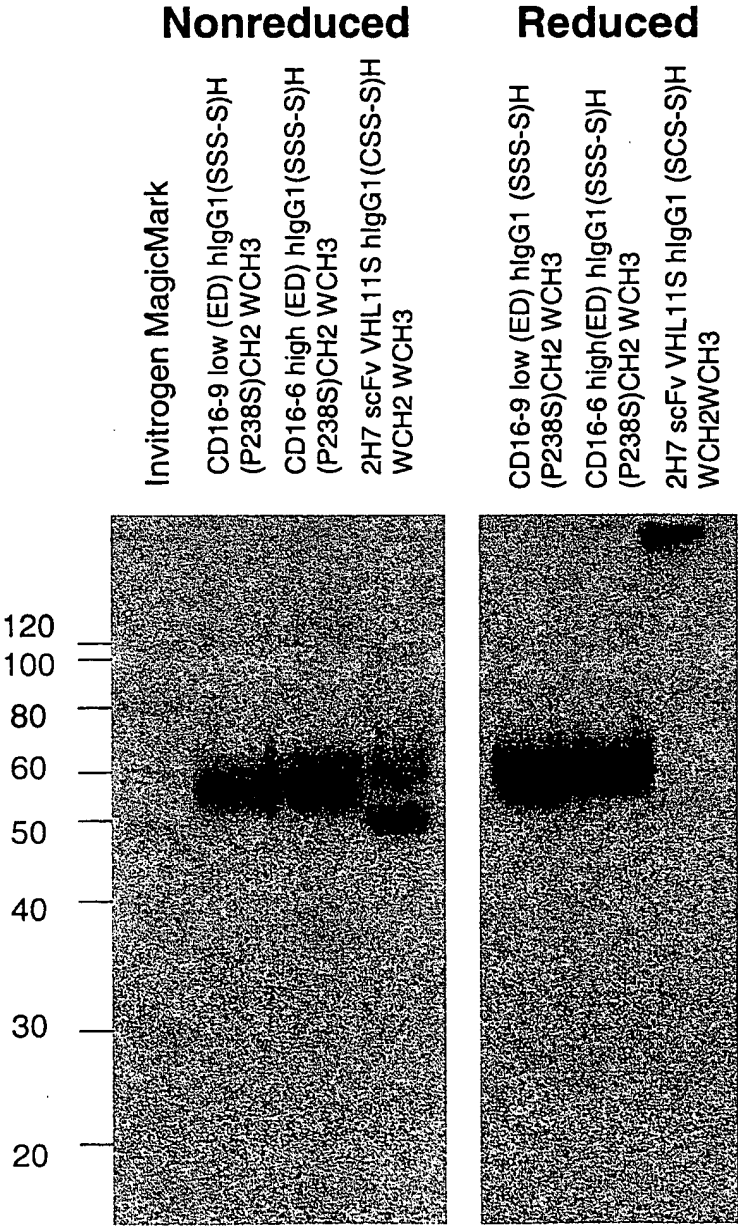


Fig. 69

Binding of soluble CD16-FITC high and low affinity fusion proteins  
to 2H7 scFv VHL11S hIgG1 (CSC-S)H WCH2WCH3 or  
(SSS-S)H (P238S)CH2WCH3 on CD20CHO Targets

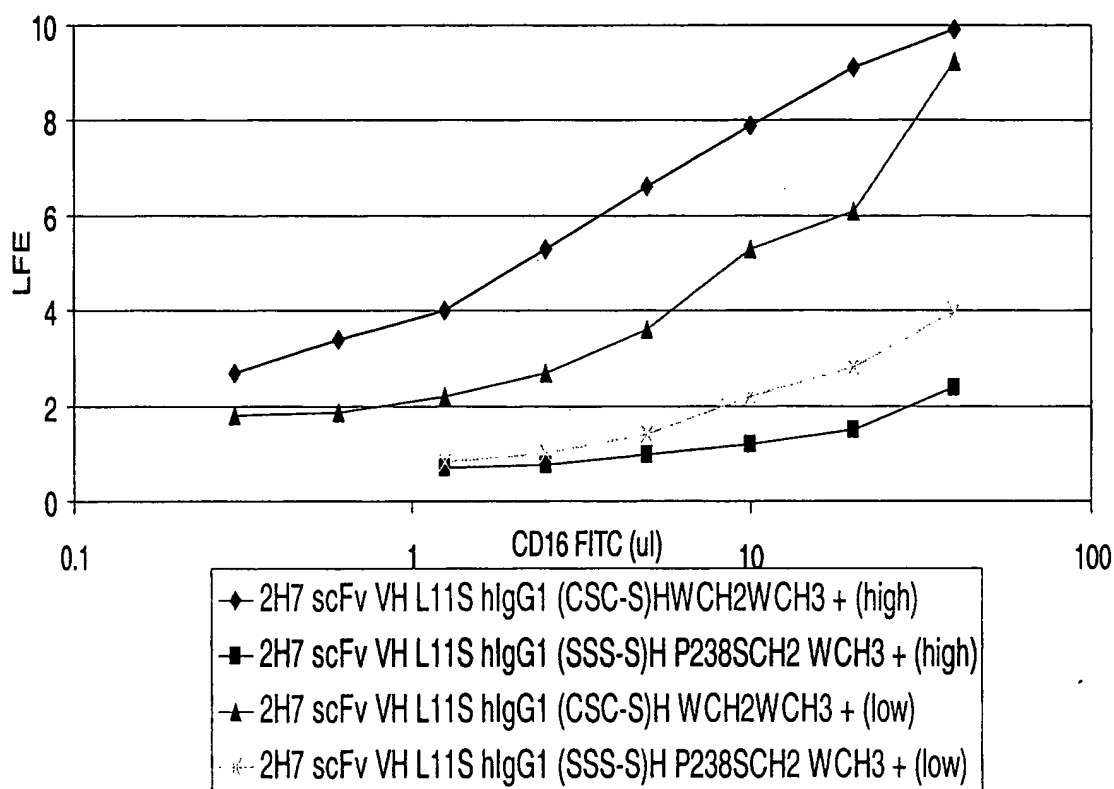
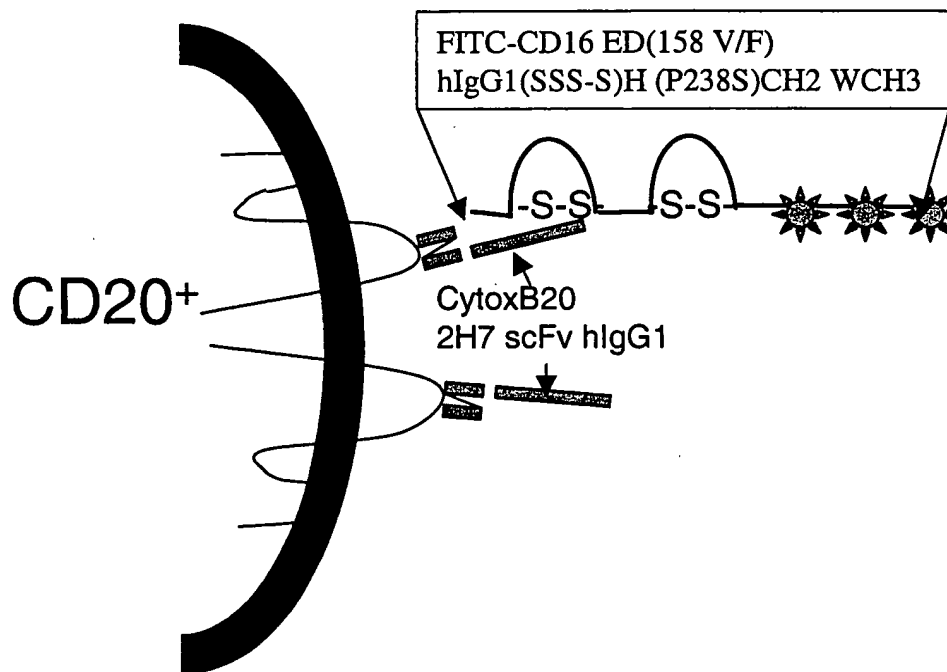


Fig. 70

Binding of FITC Labeled, Recombinant Human  
CD16(ED) extracellular domain -Ig Fusion Protein to  
CytosB Derivatives on CD20 CHO Cells



Expression of surface displayed SMIPs links  
modified cDNAs with the altered fusion proteins

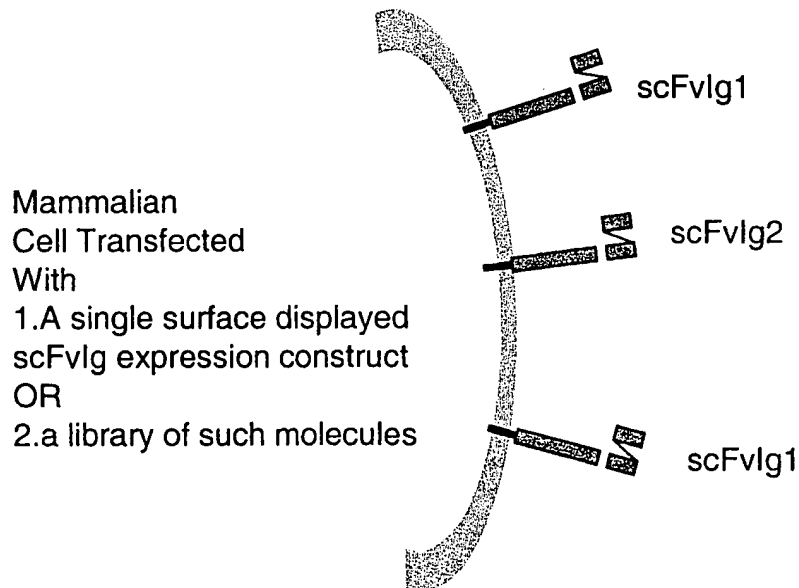


Fig. 71  
CD37 mAbs and scFvIg Induce Apoptosis

scFvIg	Bjab Staining	Annexin V Positive	
	No scFvIg	17.5	
	2H7 MH	27	
	G28-1 MH	30.6	
	G28-1 IgAH	28.9	
	HD37 MH	29.1	
	(2H7+G28-1)MH	41	
	(2H7+HD37) MH	37.1	
	(G28-1+HD37) MH	35.3	
mAbs			plus GAM
	Ramos	AnnexinV Positive	AnnexinV positive
	cells alone	3	3.3
	2H7 Mab	1.4	3.1
	G28-1 Mab	18.3	8.7
	HD37 Mab	3.7	3.1
	G28-5	3.9	8.3
	2H7+G28-1	32.3	35.7
	2H7+HD37	5	10.5
	2H7+G28-5	5.7	19.4
	HD37+G28-1	26.9	50
	HD37+G28-5	8.2	18.4
	G28-1+G28-5	39.5	68.3

### **DECLARATION OF MICHELLE HON**

I, Michelle Hon, hereby state and declare as follows:

1. On July 25, 2003, I was employed as a Summer Associate with the law firm of Buchanan Ingersoll LLP, located at 401 West A Street, Suite 1900, San Diego, California.

2. On that date, I assisted James Collett and Barbara Kielt in preparing a U.S. patent application (now assigned Serial No. 10/627,556) for Genecraft, Inc. (now known as Trubion Pharmaceuticals, Inc.).

3. At 10:00 p.m. on that date, while we were trying to print the final version of the patent application in order to take it to the Post Office, the server supporting all of the computers and printers in the office disconnected, and we lost all access to the document and were unable to continue printing. It was not until after sometime after 10:30 p.m. that the server reconnected and we were able to print the patent application.

4. The server is located in the main office of Buchanan Ingersoll PC, which is in Pittsburgh, Pennsylvania, and we had no access or control over it.

5. Prior to July 25, 2003, I have received several notifications from the Systems Department in the Pittsburgh office of Buchanan Ingersoll PC that the server would be shut down for maintenance at various times, typically over a weekend. I received no such notification that this would happen on Friday, July 25, 2003.

6. On Monday, July 28, I contacted the Systems Department in the Pittsburgh office of Buchanan Ingersoll PC to find out why the server had shut down. I was informed that it shuts down every night at 1:00 Eastern time in order to create a back-up of the system. I was informed that no notices are sent out for this nightly back-up and I surmise that this is because no one is expected to be affected at this time of night.

I declare that under penalty of perjury that the foregoing is true and correct.

Dated: May 5, 2004

A handwritten signature in black ink, appearing to read 'Michelle Hon', written over a horizontal line.

Michelle Hon

**DECLARATION OF MILDRED PAMPENA**

I, Mildred Pampena, hereby state and declare as follows:

1. On July 25, 2003, I was employed as a Manager of Networks and Communications in the Pittsburgh office of the law firm of Buchanan Ingersoll PC.
2. During July 2003, the Citrix Servers for the entire computer network of Buchanan Ingersoll PC were on a maintenance program under which the servers would automatically shut down every night at approximately 1:00 a.m. Eastern Daylight Time. During this nightly maintenance, the servers take themselves off line for maintenance, which can take up to about 30 minutes or more. During that time, all computer systems that rely on Citrix, including all computer systems and printers in the San Diego office, are unavailable to users.
3. The only way users would know that the computer systems were again available after such an automatic maintenance shut-down would be to try to log on, until they are successfully logged again.

I declare that under penalty of perjury that the foregoing is true and correct.

Dated: June, 2004

  
Mildred Pampena

**DECLARATION OF DALE STUCKEY, JR.**

I, Dale Stuckey, Jr., hereby state and declare as follows:

1. I am employed by the U.S. Postal Service at the Midway facility located on Midway Drive, San Diego. I have been employed in this position since approximately 1981.

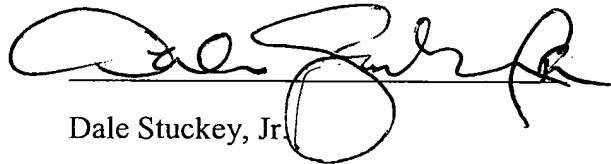
2. One of my responsibilities as a window clerk is to accept incoming mail from customers, including Express Mail packages.

3. On Friday, July 25, 2003 at approximately 11:40 p.m., Barbara Kielt, who I understand to be an Administrative Assistant employed by Buchanan Ingersoll LLP, came to the Post Office with two packages. I was advised that she wanted to send the packages by U.S. Express Mail to the United States Patent Office, and that these packages contained two patent applications.

4. While Ms. Kielt brought the packages to the U.S. Post Office at 11:40 p.m. on July 25, 2003, I did not accept them for U.S. Express Mail, or mark the "Time-In" box on the Express Mail label (Label 11-B) because the window closed at 11:00 p.m. As a result, Ms. Kielt was not able to mail her packages and obtain a date stamp indicating that she presented them for mailing to the U.S. Postal Service on Friday, July 25, 2003.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: August 27, 2003

  
Dale Stuckey, Jr.



### DECLARATION OF JOHN NORTON

I, John Norton, hereby state and declare as follows:

1. I am employed by the U.S. Postal Service at the Midway facility located on Midway Drive, San Diego. I have been employed in this position since approximately 08/01 *John*.

2. One of my responsibilities as the Supervisor: Customer Service Operations is to supervise employees at the Midway facility, and the intake of mail from customers, including Express Mail packages. At this time, I supervise approximately 20 employees at the Midway facility.

3. Dale Stuckey, Jr. is employed under my direct supervision at the Midway facility as a window clerk, and has been an employee of the Postal Service since 1981.

4. Mr. Stuckey has advised me that on Friday, July 25, 2003 at approximately 11:40 p.m., Barbara Kielt, an Administrative Assistant employed by Buchanan Ingersoll LLP, came to the Post Office with two packages. Mr. Stuckey was advised by Ms. Kielt that she wanted to send the packages by U.S. Express Mail to the United States Patent Office, and that these packages contained two patent applications.

5. Mr. Stuckey further advised me that while Ms. Kielt brought the packages to the Midway facility at 11:40 p.m. on July 25, 2003, he did not accept them for U.S. Express Mail, or mark the "Time-In" box on the Express Mail label (Label 11-B) because the window had closed at 11:00 p.m. As a result, Ms. Kielt was not able to mail her packages and obtain a date stamp indicating that she presented them for mailing to the U.S. Postal Service on Friday, July 25, 2003.

I declare under penalty of perjury that the foregoing is true and correct.

Dated: August 27, 2003

  
John Norton